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PEGGY KOERNER NITSOS for the MASTER OF SCIENCE
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Dr. R. E. Pacha

Myxobacteria are known to occur in the aquatic environment, however, little information is available regarding the taxonomy of these organisms. This investigation was initiated in an attempt to classify a group of fresh water cytophagas on the basis of biochemical and physiological as well as cultural and morphological characteristics.

The 35 isolates used in this investigation were determined to be myxobacteria on the basis of their cellular morphology. Vegetative cells were slender, gram negative, weakly refractile rods characterized by a marked flexibility. Examination of young cells revealed typical gliding motility. Cells became shorter and thicker with age and involution forms occurred. The thin, spreading, yellow to yellow-orange colonies produced by the organisms were typical of myxobacteria. Fruiting bodies were never observed although microcyst-like structures were formed by two isolates. As a result these two organisms are considered to be members of the genus Sporocytophaga.

The remaining 33 isolates can be classified in the genus Cytophaga.

The isolates were non-halophilic mesophiles which grew best at pH 7.2. All grew anaerobically under defined conditions and most were moderately thermostable. All but one of the isolates were resistant to neomycin and most were sensitive to tetracycline, streptomycin, erythromycin and novobiocin.

The results of the physiological studies showed that the myxobacteria studied in the present investigation have the capacity to degrade macromolecules. All of the organisms were lipolytic and most were proteolytic and amylolytic. Cellulolytic activity as demonstrated by the utilization of carboxymethyl cellulose was exhibited by over 70% of the isolates. All of the organisms lysed dead cells of Aerobacter aerogenes and the majority were capable of lysing dead cells of a variety of other bacteria and yeast. A small portion also lysed similar preparations of algal and protozoan cells.

Simple carbohydrates were oxidized by most of the strains, however, very few of the organisms were able to ferment these substances. Carbohydrates oxidized by the majority of the isolates include glucose, galactose, maltose and cellobiose. Of the fermentative strains, only one required substrate amounts of CO_2 for glucose fermentation. This isolate may have a metabolic pathway similar to Cytophaga succinicans, a facultative anaerobic myxobacterium which carries out a CO_2-dependent fermentation of glucose.

The nutritional requirements of the organisms were relatively

simple. The nitrogen requirement could be readily satisfied by either casein hydrolysate or KNO_3 and starch served as a sole source of carbon for most of the isolates. However, only 12 of the 35 strains were able to utilize glucose as a sole source of carbon. Simple amino acids did not support the growth of any of the isolates when used as a sole source of carbon.

The DNA base composition and carotenoid pigments of six of the isolates also were studied. Five of the organisms were found to have a DNA base composition between 34.88 and 38.54% GC. A value of 53.44% GC was found for one isolate identified as a Sporocytophaga. The results of the pigment analysis indicated that carotenoid pigments spectrally similar to lutein, alpha-carotene-5,6-epoxide and rhodopin were present in the organisms. It appears from these studies that pigment analyses of the myxobacteria could contribute significantly to the taxonomy of this group of organisms.

Based on the results of this investigation, an improved taxonomic scheme for the genus Cytophaga has been proposed. Unlike the taxonomic keys which are currently available, the major subdivisions of this scheme are based on biochemical and physiological characteristics. Key features for distinguishing members of the genus Cytophaga according to the proposed scheme include use of carboxymethyl cellulose, chitin, citrate and carbohydrates as well as nitrate reduction.

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Assistant Professor of Microbiology
in charge of major

Redacted for Privacy

Chairman of the Department of Microbiology

Redacted for Privacy

Dean of Graduate School

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INTRODUCTION

The order Myxobacterales has been defined and classified on a morphological basis. Members of this order are known to occur in soil, in marine environments and on decaying organic material. Relatively little information, however, is available regarding the occurrence, distribution and activity of the fresh water members of this unique group of organisms. Consequently, the identification of fresh water species is often difficult to ascertain.

Myxobacteria, the common name given members of the order Myxobacterales, were first described by Thaxter (86) in 1892. These bacteria are procaryotic, non-photosynthetic, gram negative unicellular rods which lack rigid cell walls and have a low index of refractility. A characteristic feature of these bacteria is their ability to hydrolyze numerous complex macromolecules such as cellulose, chitin, agar, starch and a variety of proteins. Myxobacterial organisms are also capable of lysing living and dead cell preparations of algae, bacteria, fungi and yeast. Perhaps the most unique characteristic of the members of this order is their mode of locomotion which is a creeping or gliding type of motility that occurs with an absence of flagella when the cells come in contact with a solid surface. While this particular type of motility is typical of myxobacteria it is not

confined to this group of organisms as it is also known to occur in the Beggiatoaceae, Cyanophyaceae and Vitreoscillaceae families.

The majority of the work relating to myxobacteria has been carried out on terresteral fruiting forms. The family Cytophagaceae, with which this paper is concerned, differs from the other families in the order Myxobacterales by its lack of fruiting body structures and microcysts. To date some 30 species descriptions of cytophagas have been published. Seventeen of these cytophagas were isolated from soil or wood and 10 from marine environments. Only 11 species are described in the current Bergey's Manual of Determinative Bacteriology (12). The family includes one genus which is divided into species on the basis of morphology, mode of locomotion, habitat and pigmentation. Information concerning biochemical and physiological reactions is scanty and is not included to any great extent in the species descriptions.

While fresh water non-fruiting cytophagas are known to occur, they are not included in the classification scheme provided in Bergey's Manual of Determinative Bacteriology (12). This investigation was initiated in an attempt to classify a group of fresh water myxobacterial isolates using physiological and biochemical as well as cultural and morphological characteristics in hopes that the information obtained would contribute to the taxonomy and general knowledge of this unique group of organisms.

LITERATURE REVIEW

General Description of Myxobacteria

The order Myxobacterales represents a group of bacteria which possess a number of unique characteristics. Thaxter (86) in 1892, on the basis of observations on fruiting bodies which occurred naturally on decaying wood and plant material, recognized these organisms as an independent order within the Schizomycetes. The name Myxobacteraceae was proposed for this new group. Thaxter is recognized as the first person to describe the germination of microcysts associated with these bacteria, and his work established the foundations for the present knowledge concerning fruiting myxobacteria.

The order Myxobacterales is composed of procaryotic, non-photosynthetic, gram negative protista which are able to hydrolyze a large number of insoluble macromolecules such as cellulose, chitin, starch and agar. These organisms are similar to true bacteria in that their vegetative cells are unicellular and rod-shaped. However, myxobacteria exhibit low refractility and lack rigid cell walls. The cells are non-flagellated but are capable of a creeping- or gliding-type of motility on the surface of a solid substrate. This type of motility is analogous to that found in the families Beggiatoaceae, Cyanophyaceae and Vitreoscillaceae. The precise mechanism for

this mode of locomotion has not yet been determined.

With the exception of the family Cytophagaceae, unique structures known as fruiting bodies and microcysts are characteristic of the order Myxobacterales. Fruiting bodies begin to appear at a particular stage of growth when the vegetative cells swarm together. Within the fruiting bodies some of the cells develop into resting cells or microcysts. Only the genus Sporocytophaga in the family Myxococcaceae forms resting cells without the formation of fruiting bodies. Because of the development of fruiting bodies and microcysts, higher myxobacteria are said to exhibit a type of life cycle.

Myxobacteria have been reported to occur in soil (6, 7, 30, 52), in marine environments (46, 74, 77) and on decaying organic material (70, 86). Relatively little information is available, however, regarding the occurrence, distribution and activity of these organisms in fresh water environs. Studies (3, 4, 10, 11, 64, 66, 67) concerning fresh water myxobacteria are limited primarily to organisms associated with fish (10, 11, 22, 65, 67). Particular attention has been given to two myxobacterial fish pathogens: Chondrococcus columnaris (Davis), the etiological agent of columnaris disease; and Cytophaga psychrophila (Borg), the causative agent of bacterial cold water disease.

Systematic Treatment of Myxobacteria

In the early development of myxobacterial classification, the morphology and taxonomy of myxobacteria were subject to considerable controversy. The difficulties surrounding these areas can only be surmised by taking into consideration the fact that these organisms have been placed in no less than three different orders: Spirochaetales (41), Actinomycetales (9) and, Myxobacterales (51, 52).

In 1924, Jahn (25) reported the first detailed systematic treatment of the myxobacteria. His description included four characteristics: formation of long, thin vegetative rods; production of carotenoid pigments; formation of slime; and, the formation of fruiting bodies. On the basis of fruiting structures alone, four families were recognized, with primary divisions based on the shape of the microcysts formed. The families were Myxococcaceae, with spherical or oval shaped microcysts; Archangiaceae, Sorangiaceae and Polyangiaceae, all with cylindrically shaped microcysts.

The order Myxobacterales, as now represented in Bergey's Manual of Determinative Bacteriology (12), is composed of five families. The fifth family, Cytophagaceae, was proposed by Stanier (75) and includes those myxobacteria which fail to produce fruiting bodies and microcysts. These organisms were recognized as myxobacteria on the basis of their lack of rigid cell walls, mode of

locomotion, division of cells by constriction and manner of colony growth. The family Cytophagaceae contains one genus, Cytophaga. Stanier (75) also appended the family Myxococcaceae by adding the new genus Sporocytophaga. Representatives of this group form microcysts but not fruiting bodies.

The two most important criteria which distinguish myxobacteria from the eubacteria, are the lack of rigid cell walls and the peculiar type of creeping motility exhibited by these organisms. On the basis of these features Stanier and van Niel (80) redefined the Myxobacterales in the following manner:

> Unicellular rod-shaped organisms, without rigid cell walls, which always show creeping motility (never flagella). Multiplication by transverse fission. Resting stages, if present, may be microcysts, sometimes contained within larger cysts. The individual microcysts or the larger cysts may be borne on or in fruiting bodies of various shapes.

Recent recommendations by Soriano and Lewin (73) suggest placing the non-fruiting myxobacteria, along with other gliding bacteria, including Beggiatoaceae, in a new order, Flexibacteriales. This modification would then restrict the order Myxobacterales to only those myxobacteria which possess fruiting bodies. The new order, Flexibacteriales, would consist of organisms that did not produce fruiting bodies and would include the families Beggiatoaceae with three genera; Cytophagaceae with six genera; Simonsiellaceae with two genera; Leucotrichaceae with two genera; and, an additional

small, unnamed family.

Fresh Water Myxobacteria

Probably the most thoroughly studied of the fresh water myxobacteria is Chondrococcus columnaris (Davis), a fruiting organism found to be responsible for epizootics of columnaris disease in salmon and other fishes. This organism was first successfully isolated by Ordal and Rucker (64) during an outbreak in young sockeye salmon (Oncorhymchus nerka) at the Leavenworth Hatchery, U. S. Fish and Wildlife Service. Because of the flexibility of the cells, lack of definite cell walls and peculiar creeping motility, Ordal and Rucker concluded that the organism was a myxobacterium. These myxobacterial characteristics were later confirmed through the work of Nigrelli and Hunter (60). The work of Ordal and Rucker represents the first report of animal pathogenicity among the bacteria of the order Myxobacterales.

The initial account of columnaris disease was reported by Davis (22). These outbreaks were responsible for heavy mortalities in a number of species of fresh water fishes. While unable to successfully isolate the etiological agent, Davis reported a large number of slender, motile bacteria characteristically present in lesions of infected fish. Furthermore, he found that when material from these lesions was placed in wet mounts, the bacteria collected together to

form columnar-like masses on pieces of fish tissue. Thus, he named the organism Bacillus columnaris, and the disease columnaris disease.

In an independent study, Garnjobst (32) also investigated cultures of an organism considered to be identical with those described by Davis. Due to changes in the system of classification since 1922 and on the basis of her observations on movement and lack of fruiting bodies, she excluded the bacteria from the genus Bacillus and renamed the organism Cytophaga columnaris (Davis).

Recent work (48) based on deoxyribonucleic acid (DNA) base ratios suggests that Chondrococcus columnaris might be a member of the Cytophagaceae. It is reported to have a Mole % guanine plus cytosine (GC) value of 43 which is within the accepted range for non-fruiting myxobacteria. Thus, the final decision on whether this organism should be included in the genus Chondrococcus still remains open to question and further study.

Perhaps one of the most striking observations made by Ordal and Rucker (64) was that cultures of Chondrococcus columnaris produced fruiting bodies in water. Previously, the only case of fruiting body formation in water was that reported by Geitler (33). He described submerged fruiting bodies in a myxobacterium, Polyangium parasiticum, parasitic on the alga Cladophora fracta. The vegetative cells of the latter myxobacterium are usually long and thin, with

tapering ends, and their cylindrical resting cells are borne on fruiting bodies in cysts of definite shape.

A second frequently studied fresh water myxobacterium is the etiological agent of bacterial cold water disease in fishes. This organism, originally isolated by Borg (10, 11), was found to be a non-fruiting myxobacterium unable to grow at temperatures above 25C. On the basis of its low optimal growth temperatures and its pathogenicity, Borg proposed the name Cytophaga psychrophila for this organism. His work was the first published description of a fresh water myxobacterium belonging to the cytophaga group. Descriptions of this organism have been somewhat incomplete, and it is not at present included in Bergey's Manual of Determinative Bacteriology (12).

Recently Pacha (66) reported the isolation of ten strains of Cytophaga psychrophila, obtained during a number of bacterial cold water disease outbreaks in the Pacific Northwest. Based on morphological, biochemical and serological studies, it was shown that these strains were very closely related. Results of that investigation extended the description of Cytophaga psychrophila.

Cytophaga succinicans, a nonpathogenic, non-fruiting, fresh water myxobacterium has also received considerable attention in recent years. Isolated in 1961 (4), it was described as a facultative anaerobic myxobacterium which grew anaerobically at the expense of

carbohydrate fermentation. The fermentation was said to be carbon dioxide (CO_2) consuming and perhaps even CO_2 requiring. A second paper by the same workers (3) confirmed that Cytophaga succinicans required CO_2 for glucose fermentation. They presented evidence which suggested that glucose degradation proceded via the Embden-Meyerhof pathway. The possible mechanism of CO_2 fixation and a reason for the CO_2 requirement for fermentation were also suggested.

Except for the works of Anderson and Ordal (3, 4) very little had been done on fresh water myxobacteria prior to 1968. A recent paper by Pacha and Porter (67) presents data on the morphological, cultural, biochemical and serological studies carried out on 32 non-pathogenic myxobacteria isolated from the surface of a variety of fresh water fish. The results of this work contribute considerably to the taxonomy and general information of saphrophytic myxobacteria which are not at present represented in Bergey's Manual of Determinative Bacteriology (12).

New Approaches in Bacterial Classification

A number of new approaches are now being used to supplement existing taxonomic information. These approaches include studies at the molecular level; pigment analysis; and, bacteriophage-typing. It is reasonable to assume that such information could also be used to improve the present myxobacterial classification. The following

section describes characteristic approaches which could contribute to current taxonomy.

Genetic Components

Several new approaches should be considered as means of adding further insight into the problem of myxobacterial classification. One such approach places the taxonomic study at the molecular level. This approach is based on the study of DNA: base composition and homology.

In DNA base composition analysis, strains of organisms closely resembling each other can be compared by their mean Molar (G + C) content expressed as % GC. Genera with similar properties would be expected to have the same range of % GC. Thus, DNA base composition studies can become useful tools in bacterial taxonomy to supplement morphological, physiological, biochemical and other characteristics. This is particularly true when considering that a similar DNA base ratio suggests the possibility of genetic affinities between strains as well as an eventual common phylogenetic origin (24).

Several investigations (18, 54, 55, 58) concerning the DNA base composition of myxobacteria have provided Mole % GC ranges of 68 to 71 for fruiting species while non-fruiters exhibit a range of 32 to 43 Mole % GC. The large difference in base composition and the lack of

representatives of intermediate DNA composition in taxa such as myxobacteria indicates the possibility of "missing links," and that the necessity of a reappraisal of relationships within the group is in order (58).

DNA hybridization competition experiments have been used in studying a number of myxobacteria (44). In these investigations the ratio of depression in binding caused by the test organism DNA to that obtained by homologous competitor DNA was used as an index of relatedness. Reference organisms from which labeled DNA was prepared were Cytophaga succinicans, Myxococcus xanthus strain FB and Chondrococcus columnaris. Organisms in the cytophaga group appeared to be heterogenous. Cytophaga cultures were 0 to 50% related to Cytophaga succinicans. Myxococcus xanthus strain Mla2 was 96% homologous to strain FB while Myxococcus fulvus and Myxococcus virescens are 90% homologous to strain FB. Chondrococcus coralloides showed 83% homology with Myxococcus xanthus. Chondrococcus columnaris was shown to lack homology with Chondrococcus coralloides, other higher myxobacteria and cytophagas. DNA homology between cytophaga groups and higher myxobacteria could not be demonstrated by these workers.

Pigmentation

Chromogenesis can also be an important determinative trait used for the systematic classification of bacteria. Myxobacteria are generally orange or yellow in color although a few are white, pink or olive green. The genus Myxococcus is classified largely on the basis of pigmentation, just as species are differentiated in the genus Cytophaga. A systematic analysis of the pigments found in myxobacteria under standardized conditions can provide additional biochemical information helpful in the evaluation of possible affinities among the organisms studied.

Jahn (25) suggested that the pigments of myxobacteria were carotenoid in nature. Subsequent analysis of pigment formation in myxococci has confirmed this (13, 34, 35, 36). Work by Anderson and Ordal (4) indicates that much or all of the pigments of Cytophaga succinicans reside in the cell wall and that these pigments are carotenoid in nature exhibiting an absorption maxima at 475 and 450 mμ with an inflexion at about 420 mμ.

Bacteriophage

The susceptibility of a bacterium to lysis by a bacteriophage, or the ability of a bacterium to form a lysogenic system with a bacteriophage, also represent useful taxonomic tools (82). A particular phage

will attack and lyse only a characteristic, limited range of bacterial strains. The phage-sensitivity of a strain as a basis for bacterial classification has been interpreted in two ways (59). Phage-sensitivity can represent a phenotypic character which two bacterial strains may have in common. If phage-sensitivity is represented at the genetic level then two bacterial strains which interact with the same phage at the same genetic level manifest the same degree of genetic compatibility with the phage, and thus with each other. The major use of phage-typing is to distinguish between closely related bacterial strains which cannot be distinguished in other ways. Excellent examples of widely used phage-typing systems are those for Salmonella typhi and Staphylococcus aureus.

The first report of a bacterial virus for a member of the Myxobacterales was provided by Anaker and Ordal (2). These workers described a bacteriophage against Chondrococcus columnaris. Since then other investigators have isolated and characterized bacteriophage which attack this same species (48, 49) as well as other myxobacterial species (13, 74, 87).

MATERIALS AND METHODS

Isolates

Thirty-five different cultures were studied in this investigation. Of these, 33 were previously isolated by Dr. R. E. Pacha from fresh water streams in the Corvallis, Oregon area. These cultures had been lyophilized and were available for study. A lyophilized culture of Cytophaga succinicans (RL-8) was kindly supplied by Dr. E. J. Ordal of the University of Washington, Seattle, Washington. This culture had been isolated from a chinook salmon fingerling at the University of Washington hatchery. An additional organism was isolated by the author during the spring of 1968 from Marys River, south of Philomath, Oregon. Table 1 provides a list of the organisms, together with their source and date of isolation.

Media

Cytophaga medium was routinely used for the growth of cells for the various physiological and biochemical tests, and for maintaining stock cultures. The composition of this medium, as well as other media used in this investigation, is listed in the Appendix.

Table 1. Isolates Used in Investigation.

Code Number	Culture Designation	Year Isolated	Source
1	1BCr-66-M9-1	1966	Berry Creek
2	1BCr-66-M9-3	1966	Berry Creek
3	1BCr-66-M9-4	1966	Berry Creek
4	1BCr-66-M9-5	1966	Berry Creek
5	1BCr-66-M9-8	1966	Berry Creek
6	1BCr-66-M9-10	1966	Berry Creek
7	1BCr-66-M9-11	1966	Berry Creek
8	1BCr-66-M9-12	1966	Berry Creek
9	3BCr-66-M6-1	1966	Berry Creek
10	1-OC-4-66-1	1966	Oak Creek
11	1-OC-4-66-2	1966	Oak Creek
12	1-OC-4-66-3	1966	Oak Creek
13	1-OC-5-66-1	1966	Oak Creek
14	1-OC-5-66-2	1966	Oak Creek
15	1-OC-5-66-3	1966	Oak Creek
16	1-OC-7-66-2	1966	Oak Creek
17	1-OC-7-66-3	1966	Oak Creek
18	1-OC-7-66-4	1966	Oak Creek
19	1-OC-8-65-1	1965	Oak Creek
20	1-OC-8-65-2	1965	Oak Creek
21	1-OC-8-65-3	1965	Oak Creek
22	1-OC-8-65-4	1965	Oak Creek
23	1-OC-8-65-5	1965	Oak Creek
24	2-OC-5-66-1B	1966	Oak Creek
25	2-OC-5-66-2B	1966	Oak Creek
26	2-OC-5-66-3	1966	Oak Creek
27	2-OC-5-66-4	1966	Oak Creek
28	2-OC-6-66-2	1966	Oak Creek
29	2-OC-6-66-3	1966	Oak Creek
30	2-OC-6-66-4	1966	Oak Creek
31	2-OC-6-66-5	1966	Oak Creek
32	3-OC-8-66-1	1966	Oak Creek
33	2-OC-6-66-1	1966	Oak Creek
34	*Cytophaga succinicans* (RL-8)	1957	Univ. Washington hatchery
35	UP-1-68	1968	Marys River

Culture Maintenance

Stock cultures were maintained through serial transfers in Cytophaga agar deeps. Cultures were incubated at 27C from two to five days and then stored at 5C until used. Transfers were routinely made every 30 days.

Difficulty was encountered in growing cultures 16 and 28 in liquid Cytophaga medium. It was found that these organisms grew best in casein hydrolysate broth. However, maintaining stocks of these cultures using Cytophaga agar deeps presented no problem.

Morphology

Cellular Morphology

Kopelloff's modified Gram's stain was applied to air dried smears made from 24 hour broth cultures grown at 27C. These slides were examined under a light microscope in order to determine the gram reaction of the cells.

Cell morphology and gliding motility were determined by examining wet mounts of 24 hour vegetative cells by means of phase contrast microscopy. An eyepiece micrometer was used to determine cell size. Gliding motility was also observed by examining the edge of a colony growing on Cytophaga agar using the high-dry objective of a phase contrast microscope.

Colony Morphology

Colony morphology was determined by examining cultures growing on Cytophaga agar, peptonized milk agar (43) and 1/10 Cytophaga peptonized milk agar. Observations were made at intervals from 12 to 72 hours after incubating at 27C. Colony edges were examined with both stereoscopic dissecting and phase contrast microscopes.

Fruiting Body Formation

Fruiting body formation was determined by inoculating rabbit dung pellets embedded in non-nutrient agar (1.5% (w/v) Difco agar) with cells from 48 hour plate cultures. These rabbit dung plates were then incubated at 27C. In an alternate method, dead Escherichia coli cells were streaked onto the surface of non-nutrient agar plates. One end of the streak was then inoculated with the test organism and the plates were incubated at 27C. Observations for fruiting bodies in both methods were made at intervals over the 30 day incubation period.

Microcyst Formation

The presence of microcysts was determined by observing wet mounts of cells grown in a mineral salts medium containing cellulose powder (0.5%; w/v). Sterile medium was dispensed in 50 ml aliquots

into either 250 ml Erlenmeyer flasks or 8 oz bottles. The medium was inoculated with 0.2 ml of washed cells which had been grown in broth for 24 hours and subsequently incubated at 27C on a shaker for 30 days.

Environmental Characteristics

Anaerobic Growth

The ability of the test organisms to grow anaerobically was determined using the anaerobic medium of Anderson and Ordal (4). The glucose and sodium bicarbonate were filter sterilized and added aseptically to the tubes. Tubes, having a final volume of 10 ml, were steamed to remove excess oxygen, cooled and inoculated with 0.2 ml of a broth culture. Sterile vaspar was then layered over the tubes and overlayed with 1% (w/v) Ion agar No. 2. Turbidity was determined visually at 24 and 48 hours, and at one week.

Resistance Tests

Sensitivity of the organisms to antibiotics was determined using Difco sensitivity discs. The isolates were tested for their susceptibility to the following antibiotics: polymyxin B, 50 units; penicillin G, 2 units; bacitracin, 2 units; streptomycin, 10 μg; novobiocin, 5 μg; erythromycin, 2 μg; gantrisin, 50 μg; neomycin, 5 μg; tetracyclin, 5 μg. Cytophaga agar plates were seeded by

overlaying with 3 ml of 0.7% Cytophaga agar containing 0.2 ml of broth culture. After the overlay agar was gelled, the sensitivity discs were aseptically applied to the surface and the plates incubated at 27C. Inhibition, represented by a clear zone around the discs, was checked at 24 and 48 hours.

The heat resistance of 48 hour vegetative cells was tested using 5 ml Cytophaga broth tubes inoculated with 0.2 ml of 48 hour broth culture. Survival of these cultures was determined after exposure to: 37C for 5 minutes; 37C for 15 minutes; 55C for 5 minutes; and, 55C for 15 minutes. After the appropriate exposure time, the tubes were cooled in ice and incubated at 27C. Turbidity was determined visually at 24 and 48 hours, and at one week.

Thirty day old cells were also subjected to heat treatments. Tubes containing 5 ml of Cytophaga broth were inoculated with 0.2 ml 24 hour broth cultures and incubated for 30 days at 27C. At this point 0.2 ml of the 30 day cultures were reinoculated into 5 ml of fresh Cytophaga broth. Both the newly inoculated Cytophaga broth and the 30 day tubes were held at 50C for 5 minutes; 50C for 15 minutes; 70C for 5 minutes; and, 70C for 15 minutes. Aliquots of these cultures were then inoculated onto Cytophaga agar plates and colony development checked at intervals up to one week. The newly inoculated tubes were also incubated and checked visually for turbidity during the same time interval.

Biochemical Characteristics

Carbohydrate Utilization

The medium employed to determine if carbon dioxide was required for glucose fermentation consisted of the anaerobic medium described by Anderson and Ordal (4) supplemented with 0.0015% bromthymol blue. Tubes were steamed to remove excess oxygen, cooled and inoculated. The tubes were then sealed by layering on sterile vaspar and overlaying with 1% Ion agar No. 2. The presence of acid, represented by a color change from green to yellow, was noted at 24 and 48 hours, and at one week.

Porter's modification (68) of Hugh and Leifsons' (40) procedure was used to determine acid production from glucose, lactose, sucrose, galactose, maltose, cellobiose and mannitol. These carbohydrates were filter sterilized and added aseptically to a final concentration of 0.5%. The presence of acid was noted at 24 and 48 hours, and at one and two weeks after inoculation.

Macromolecule Utilization

Unless otherwise stated, Cytophaga agar was used as the basal medium to test the ability of the isolates to degrade starch, gelatin, casein, and tributyrin. The inoculum consisted of 24 to 48 hour broth cultures and incubation of the inoculated test media was carried

out at 27C for 24 to 48 hours.

Starch hydrolysis was detected by using basal medium containing 0.2% (w/v) soluble potato starch. After inoculation and incubation, the plates were flooded with Gram's iodine solution. The flooded plates were observed for clear zones around colony growth in an otherwise blue-black field.

Gelatinase production was tested using the basal medium supplemented with 0.4% (w/v) gelatin. After incubating, the agar surface was flooded with acid mercuric chloride (29). Presence of clear zones around colony growth indicate areas of gelatin liquification.

Casein hydrolysis was detected by inoculation of the test organisms onto basal medium containing 2% (v/v) skim milk. Plates were examined for the development of clear areas in the opaque medium as positive evidence for hydrolysis.

Tributyrin hydrolysis (21) was noted by a clearing produced on basal medium containing 0.2% (v/v) tributyrin. Plates were read at intervals up to two weeks.

The ability of the test organisms to hydrolyze aesculin was examined by inoculating ferric ammonium citrate medium (69) containing 0.1% (w/v) Difco aesculin. Plates were examined at 24 and 48 hours, and at one week, for a blackening of the medium around colonies.

Chitin degradation was determined in the following manner:

non-nutrient agar plates were overlayered with 5 ml of chitin agar. The chitin was prepared according to the procedure by Stanier (78). The plates were dried overnight at 32C and the overlay was then spot inoculated with 48 hour plate cultures. The overlay was examined for dissolution of the chitin around colony growth at intervals to 30 days.

A modification (14) of a procedure by Emerson and Weiser (26) was used to prepare carboxymethyl cellulose medium. The dry carboxymethyl cellulose powder was added slowly to distilled water in a Waring blender. When the powder was thoroughly wetted, the remaining components were added. Bromthymol blue was added to aid in detecting depressions around colony growth. Plates were poured, dried overnight at 32C and inoculated with broth cultures. The surface of the plates were observed at 24 and 48 hours, and at one week for signs of depressions in the medium around colony growth indicating the ability of the test organisms to utilize this substrate.

Utilization of cellulose powder by the test organisms was determined by using mineral salts medium supplemented with 0.5% (w/v) cellulose powder. Fifty ml aliquots were dispensed into 250 ml Erlenmeyer flasks or 8 oz bottles, inoculated with 0.2 ml washed 24 hour broth cultures and incubated on a shaker. The medium was checked visually at intervals up to 30 days for the disappearance of the cellulose powder.

Miscellaneous Physiological Tests

Unless otherwise stated, a 0.2 ml inoculum of 24 hour broth cultures was used. Incubation was carried out at 27C and the results were checked at 24 and 48 hours.

Catalase activity was determined by adding one or two drops of 3% H_2O_2 onto the surface of plate cultures. Gas bubbles are indicative of the presence of the catalase enzyme.

Tryptophanase activity was measured by indol production from a medium high in tryptophan. Tryptone broth (1%; w/v) was inoculated and, after appropriate incubation, 0.2 ml Kovac's reagent was layered over the surface of the test medium. A cherry red color at the interface within a few minutes indicates the presence of indol.

A modification of Clark-Lub medium was used for the methyl red and Voges-Proskauer tests. A positive methyl red test is evidenced by the presence of a red color after the addition of ethanolic methyl red, prepared as directed in the Manual of Microbiological Methods (71). A positive Voges-Proskauer test is indicated by a red color development within 30 minutes after adding 0.2 ml each of 40% KOH and alpha-naphthol.

The presence of H_2S was determined using 0.4% (w/v) tryptone as the growth medium. After the tubes were inoculated, strips of lead acetate paper were inserted to just above the surface of the liquid.

The strips were checked at intervals up to two weeks for the presence of a black precipitate.

Litmus milk was prepared as directed in the Manual of Microbiological Methods (71). Tubes were observed at intervals up to 30 days.

The ability of the test organisms to reduce nitrate broth aerobically and anaerobically was tested using a modified (68) nitrate broth medium. The tubes that were to be incubated under anaerobic condtions were steamed and cooled prior to use. After inoculation, these tubes were sealed with sterile vaspar, overlayed with 1% Ionagar No. 2 and then placed in a BBL #06-200 GASPAK anaerobic jar. After incubation, 0.2 ml each of sulfanilic acid and dimethyl-alpha-naphthylamine were added to the tubes. A red color development indicated those test organisms capable of reducing nitrate. False negatives were determined by adding trace amounts of zinc powder to tubes showing no color reaction. Tubes that produce a red color after the addition of the zinc powder indicate the nitrate present was not reduced by the test organisms. However, tubes that remain colorless following zinc treatment indicate those organisms which were able to reduce the total nitrate to ammonia or gaseous nitrogen. Inverted Durham fermentation tubes were used to collect any gaseous nitrogen produced.

Denitrification was determined using Burnison's modification

(15) of a medium developed by Stanier, Palleroni and Doudoroff (81). Tubes were checked for turbidity and gas production 18 and 24 hours after inoculation, and at one week.

A modification of Christensen's procedure (16) was used to detect urease activity among the test organisms. The urease broth contained basal medium supplemented with 0.1% glucose and 0.1% urea. Controls consisted of basal medium and basal medium having either 0.1% glucose or 0.1% urea. These controls were utilized to detect false positive reactions and to ascertain whether the production of acid from the glucose would lead to false negative results. The medium was filter sterilized and dispensed aseptically. After incubation, a drop of phenol red indicator was added to determine urease activity. A deep red color represented a positive reaction.

Tyrosine degradation was determined by the disappearance of tyrosine (0.5% (w/v) incorporated in Cytophaga agar) within two weeks (21).

Porter's modification (68) of a procedure reported by Niven, Smiley and Sherman (61) was used to detect the ability of the test organisms to produce ammonia from arginine. The arginine medium was filter sterilized, dispensed aseptically into sterile test tubes and inoculated. The presence of ammonia was determined quantitatively at intervals up to two weeks by adding one drop of culture to one drop of Nessler's reagent in a spot plate.

Lysine decarboxylase activity was tested using a modification (68) of the medium described by Carlquist (15). Duplicate sets of screw cap tubes containing a final volume of 5 ml were set up. One set of tubes contained 0.5% filter sterilized lysine per 5 ml volume. After incubating 18 to 24 hours, 1 ml of 4N-NaOH was added and the tubes thoroughly mixed. Two ml of chloroform were then added followed by vigorous shaking. Cadaverine, when present, is found in the chloroform phase after extraction. The tubes were then centrifuged for 10 minutes at 10,000 rpm to break the emulsion and 0.5 ml of the clear chloroform extract was removed and placed in a 13 x 100 mm test tube. Five-tenths ml of 0.1% ninhydrin (1,2,3-triketohydrindene) in chloroform was then added. The development of a deep purple color within four minutes indicates a positive reaction.

The cytochrome oxidase test of Gaby and Hadley (31) was employed to detect the presence of this enzyme in the test organisms. After inoculation and incubation, 0.2 ml of 1% alpha-naphthol in 95% ethanol and 0.3 ml of 1% aqueous p-amino-dimethylaniline oxalate (Difco) were added. Immediately after the addition of the reagents, the tubes were shaken vigorously to insure mixing and thorough oxygenation of the culture. The appearance of a blue color (indophenol blue) within two minutes after shaking is indicative of the presence of cytochrome oxidase in the cells. The reagents were prepared fresh weekly to insure valid results. Oxidase activity may also be detected

on plate cultures. In this case, equal amounts of the two reagents are mixed and several drops allowed to flow over isolated colonies.

Nutritional Studies

Unless otherwise stated, Stanier's mineral basal medium (75), supplemented with various carbon and nitrogen sources to a final concentration of 0.1%, was used in the nutritional studies. In all cases the cells were washed three times prior to initial inoculation into test media. Inoculated tubes were incubated at 27C for a period of one week and a one loop transfer was then made from all tubes showing growth. Growth in the second tubes was then used as evidence for the utilization of a particular substrate as a sole source of carbon or nitrogen.

The ability of the test organisms to utilize casein hydrolysate, KNO_3, $(NH_4)_2SO_4$ and KNO_2 as sole sources of nitrogen was tested using the basal medium containing 0.2% (w/v) soluble potato starch as the carbon source. The ability of the test organisms to grow in the presence of atmospheric nitrogen was also checked using the basal medium containing 0.2% soluble starch.

The utilization of glucose as the sole source of carbon was examined using the basal medium containing 0.1% KNO_3 as the nitrogen source. Filter sterilized glucose was added at concentrations of 0.5%.

Various other organic and inorganic substrates were tested to

determine whether or not they could be used as sole sources of carbon. The compounds tested included cellobiose, proline, glutamic acid, glycine, alanine, cysteine, arginine and sodium bicarbonate. Citrate utilization was tested using Koser's citrate medium (50).

Lytic Properties

The ability of test organisms to lyse dead cell preparations of algae, bacteria, a protozoan and a yeast was examined. The substrate cells used in this study were harvested from two to three day broth cultures, washed three times with distilled water and resuspended in 160 ml distilled water. Saline (0.85% NaCl; w/v) was used to wash the protozoan cells. Difco agar (1.5%; w/v) was added and the preparation autoclaved at 15 psi for 45 minutes.

Non-nutrient agar plates were dried overnight at 32C and then overlayed with 5 ml of dead cell agar. The plates were again dried overnight and the overlay was then spot inoculated using 48 hour plate cultures of the myxobacteria. The overlay was checked for areas of lysis at intervals up to one week.

Cultures used in preparing dead cell agar included Anabaena sp.; Scenedesmus obliqus strain D_3; Arthrobacter sp.; Corynebacterium hofmanii; Alcaligenes viscolactis; Sarcina ureae; Pseudomonas aeruginosa; Bacillus megatherium; Bacillus subtilis; Aerobacter aerogenes; Escherichia coli; Streptococcus faecalis; Staphylococcus

aureus; Mycobacterium smegmatis; Proteus vulgaris; Euglena gracilis; and, Saccharomyces cerevisiae.

DNA for Thermal-Denaturation

The DNA used in these studies was isolated from cultures 4, 8, 20, 25, 33 and 34 using the method of Marmur (56). Four to six grams of cells were lysed with sodium lauryl sulfate.

DeLey and Schell (23) discussed the procedure and instrumentation that has been used to determine thermal melting points (Tm) of purified DNA. In the present investigation samples were diluted to a final absorbance reading of 0.2 to 0.4 at 260 mμ. Two and one-half ml of the sample were placed into each of two stoppered standing cuvettes (Precision Cells, Inc.). Two and one-half ml of standard saline-citrate was used as a blank and 2.5 ml calf thymus DNA was used as a control.

The four cuvettes were placed in a holder and then into the sample chamber of a Beckman DU (Model 2400) spectrophotometer equipped with a multiple sample absorbance recorder (Model 2000, Gilford). After allowing the samples to equilibrate at 25C, absorbancy readings were recorded. The temperature was then raised to 60C and allowed to equilibrate. The temperature was then increased approximately 1C every two minutes and absorbancy readings recorded until no further increase in absorbancy was noted.

The absorbancy readings were corrected for thermal expansion of the liquid by multiplying the values by the ratio V_t/V_{25C}, where V_t equals the volume of water at temperature t and V_{25C} is the volume of the same water at 25C. These values were in turn expressed as relative absorbance by multiplying each by the reciprocal of the absorbancy reading at 25C.

A plot of relative absorbance values on the y-axis and temperature on the x-axis results in a sigmoidal curve. The mid-point of the curve is found by determining the temperature at the beginning and end of the melt. The average of these two temperatures is the mid-point or Tm of the curve. The base ratios of the DNA samples were calculated from the average Tm of two determinations as obtained from thermal denaturation curves. The formula of Marmur and Doty (57) was used to calculate the G+C content of the samples: $Tm = 69.3 + 0.41\ (G+C)$.

Pigment Analysis

An attempt was made to isolate and characterize the pigments of cultures 4, 8, 20, 25, 33 and 34. Cells were cultured, harvested and washed three times with distilled water. Five hundred microliters of each strain were subjected to methanol extraction according to the procedure described by Cooney, Marks and Smith (19). A few crystals of Tenox BHT (butylated hydroxytoluene, Eastman Kodak

Company) were added to the cells prior to pigment extraction to prevent the oxidation of the pigments.

Distilled water was added to the crude extract to dilute the methanol to 90% (v/v). This preparation was then partitioned against petroleum ether. Saturated aqueous sodium chloride was added to facilitate phase separation. This procedure was repeated until the ether phase was colorless.

Epiphasic (ether) fractions were pooled, dried over anhydrous Na_2SO_4 and evaporated to dryness under a stream of nitrogen. The residue was dissolved in a small quantity of anhydrous diethyl ether and applied to a silica gel G column (2.5 x 8 cm). The column was eluted with diethyl ether followed by methanol. The fractions obtained from the respective elutants were evaporated to dryness under a stream of nitrogen and the residue dissolved in a small amount of chloroform: methanol (2:1; v:v) containing a few crystals of Tenox BHT. These solutions were spotted onto silica gel G thin layer chromatography plates in a thin layer box through which a steady flow of nitrogen was passed. The plates were developed in benzene: absolute methanol: glacial acetic acid (87:11:2; v:v:v) containing a few crystals of Tenox BHT after which the plates were dried in a nitrogen atmosphere. The pigment bands were removed, eluted with methanol and evaporated to dryness under a stream of nitrogen.

Absorption spectra (550 to 350 mμ) were determined by using a

Beckman DK-2A ratio recording spectrophotometer. Spectra were made by suspending the eluted pigments into each of the following solvents: hexane; chloroform; and, carbon disulfide. Spectral maxima obtained were then compared with recorded absorption maxima of various known carotenoids (20).

Bacteriophage Assay

The existence of bacteriophages for a number of test organisms was also examined. Cultures 2, 9, 17, 19, 21, 30, 32 and 34 were incubated overnight at 27C in tryptone-calcium chloride broth. These cultures were then subcultured in the same medium and re-incubated.

A 24 hour enrichment was prepared by adding 1 ml of an overnight broth culture and 1 ml of water obtained from Oak Creek to 30 ml of tryptone-calcium chloride broth. After incubating overnight at 18C the enrichment was centrifuged at 10,000 rpm for 10 minutes in sterile tubes and the supernatant collected. This supernatant was then assayed for the presence of phage by using the conventional agar layer method described by Adams (1) in which 0.1 ml supernatant and 0.3 ml of an overnight tryptone-calcium chloride culture were used. The overlay was examined for plaque formation at 24 and 48 hours after incubation at 18C.

Plates which showed evidence of plaque development were

harvested by adding 5 ml of tryptone-calcium chloride broth to the surface of the plate and scraping the overlay. This was transferred to sterile tubes, centrifuged 10 minutes at 5000 rpm to remove the agar and the supernatant (phage stock) collected in sterile bottles. Repeating the overlay agar method several times yielded a relatively high titer. The stock was then Millipore filtered in order to obtain a bacterial-free phage preparation. This preparation was then serially diluted and plated to determine the phage titer and to determine the actual presence of phage rather than bacteriocin-like substances. The purified stocks were stored at 8C in tryptone-calcium chloride broth.

RESULTS

Cell Morphology

Microscopic examination of young vegetative cells of the fresh water isolates used in this investigation revealed typical myxobacterial features. The vegetative cells were found to be slender, gram negative, weakly refractile rods with rounded ends and were characterized by a marked flexibility. The length of young cells ranged from 4 to 7 μ and their width from 0.36 to 0.47 μ. As the cultures aged the cells tended to become shorter and thicker. Older cells exhibited lengths of 2 to 4 μ and widths of 0.5 to 1.0 μ. It was also noted that the degree of flexibility exhibited by the cells decreased with age. In extremely old cultures, 30 days or older, the cells appeared rigid which is characteristic for most true bacteria. Similar changes in cellular morphology have been reported by Stanier (78).

Cell form or shape was also affected by age. In broth cultures, three weeks or more in age, involution forms similar to those described by Garnjobst (32) were noted. These involution forms consisted of curved, coiled and ring, or oval forms varying in size and shape.

Motility

An examination of young vegetative cells in wet mounts revealed a slow, even, gliding-type of motility. This type of motility differs from the rapid directional changes associated with motile flagellated microorganisms. Gliding movement is dependent upon contact between the cells and a solid surface and occurs in the absence of flagella. Periodically one end of the vegetative cell becomes attached to the surface of the cover glass and the other end flips up and oscillates back and forth simulating a swinging motion.

The rate of movement appears to vary widely with the different cultures. This rate has been measured by some investigators (42, 79) and has been found to be as much as 15 μ per minute. As the cells age motility decreases. Some older cells may still maintain the flexing and swinging movements but this is unusual.

Colony Morphology

Swarming colonies were produced by the majority of the isolates when grown on Cytophaga agar. Colonies were thin, translucent and flat, and tended to be spreading in nature. This was especially true when the cells were grown on the more dilute 1/10 Cytophaga peptonized milk agar medium. The edges of most of the colonies were quite irregular and appeared to be composed of a thin

mono-layer of cells when examined under the phase contrast microscope. Only two of the cultures, 26 and 28, appeared to have smooth entire edges. Culture 33 developed a peculiar colony morphology after about three days of incubation. There appeared to be a local accumulation of cells in drop-like masses surrounding the major portion of the colony and the typical irregular edge was usually absent.

The colonies were irridescent when examined under reflected light. Coloration was predominately yellow or yellow-orange. Culture 33, however, was pink and cultures 20, 30 and 31 were white. Culture 25 produced a brown diffusible pigment after two weeks of growth in a Cytophaga agar deep.

Fruiting Body and Microcyst Formation

Since the classification scheme for myxobacteria as presented in Bergey's Manual of Determinative Bacteriology (12) places much emphasis on the presence or absence of fruiting body structures and microcysts, an attempt was made to determine the presence of these structures in the isolates used in this investigation. Fruiting body production could not be detected in any of the 35 isolates examined. The drop-like masses characteristically found in culture 33 appeared at first to be similar to immature fruiting bodies, but examination revealed normal vegetative cells. Microcyst-like structures appeared to occur in culture 4 and possibly culture 20. When agar block

preparations of culture 4 were examined over a period of six hours, microcyst germination was found to occur. The structures in culture 4 appeared to be refractile spheres when observed under a phase contrast microscope. In culture 20, the cells appeared to merely shorten and thicken to a point of being quite coccoidal. These shortened cells could be seen to elongate when examined in slide culture by phase contrast microscopy.

Cultural Responses

Temperature Effects on Growth

The ability of the isolates to grow at temperatures ranging from 5C to 55C was tested and the results are represented in Table 2. Optimum growth of the 35 isolates was obtained at 18C and 27C. Growth at lower temperatures occurred, but the rate was considerably slower. At 30C, 31 of the cultures produced slight growth, and temperatures above 30C were inhibitory for all cultures. Since all of the isolates were able to grow at 25C and above, they have been considered to be mesophiles.

Effects of pH on Growth

Cytophaga broth, buffered with either acetate or phosphate, was used to determine the effects of pH on the growth of the isolates.

Table 2. Effects of Temperature on the Growth of the Isolates.

	Temperature								
Strain	5C	8C	10C	18C	27C	30C	37C	45C	55C
1	+	++	++	+++	+++	-	-	-	-
2	+	++	++	+++	+++	±	-	-	-
3	+	++	++	+++	+++	+	-	-	-
4	+	++	++	+++	+++	+	-	-	-
5	+	++	++	+++	+++	-	-	-	-
6	+	++	++	+++	+++	±	-	-	-
7	+	++	++	+++	+++	+	-	-	-
8	+	++	++	+++	+++	+	-	-	-
9	+	++	++	+++	+++	+	-	-	-
10	+	++	++	+++	+++	+	-	-	-
11	+	++	++	+++	+++	+	-	-	-
12	+	++	++	+++	+++	+	-	-	-
13	+	++	++	+++	+++	+	-	-	-
14	+	++	++	+++	+++	+	-	-	-
15	+	++	++	+++	+++	+	-	-	-
16	+	++	++	+++	+++	+	-	-	-
17	+	++	++	+++	+++	+	-	-	-
18	+	++	++	+++	+++	+	-	-	-
19	+	++	++	+++	+++	+	-	-	-
20	+	++	++	+++	+++	+	-	-	-
21	+	++	++	+++	+++	+	-	-	-
22	+	++	++	+++	+++	+	-	-	-
23	+	++	++	+++	+++	+	-	-	-
24	+	++	++	+++	+++	+	-	-	-
25	+	++	++	+++	+++	+	-	-	-
26	+	++	++	+++	+++	+	-	-	-
27	+	++	++	+++	+++	+	-	-	-
28	+	++	++	+++	+++	+	-	-	-
29	+	++	++	+++	+++	+	-	-	-
30	+	++	++	+++	+++	+	-	-	-
31	+	++	++	+++	+++	+	-	-	-
32	+	++	++	+++	+++	+	-	-	-
33	+	++	++	+++	+++	+	-	-	-
34	+	++	++	+++	+++	+	-	-	-
35	+	++	++	+++	+++	+	-	-	-

+: Slight Growth.
++: Moderate Growth.
+++: Abundant Growth.
-: No Growth.
±: Growth Questionable.

These results are presented in Table 3. The cultures were grown at pH values ranging from 5.0 to 9.0. Data indicate a greater tolerance to alkaline conditions than to acid conditions. Sixteen of the 35 cultures examined were able to initiate growth at pH 9.0 but none were able to grow at pH 5.0 or 5.5. All of the isolates were capable of initiating growth at pH 7.2.

Effects of Sodium Chloride on Growth

To determine the extent of tolerance of the isolates to salt, sodium chloride was added to Cytophaga broth in concentrations ranging from 0.2% to 3.0%. The data, summarized in Table 4, indicate that all cultures were capable of growth in the absence of the salt. This suggests that the Cytophaga broth medium employed contains sufficient sodium and chloride ions to allow for growth. With the addition of increasing concentrations of sodium chloride, the number of organisms capable of growth decreased. At 0.2% salt, 33 of the isolates were able to grow while at 1.0%, 26 cultures exhibited moderate growth. Concentrations of 3.0% sodium chloride were inhibitory for all the isolates. These findings indicate that the isolates are not obligate halophiles.

Table 3. The Effect of pH on the Growth of the Isolates.

	pH								
Strain	5.0	5.5	6.0	6.5	7.0	7.2	7.5	8.0	9.0
1	-	-	+	+	+	+	+	+	-
2	-	-	+	+	+	+	+	+	+
3	-	-	+	+	+	+	+	+	+
4	-	-	+	+	+	+	+	+	+
5	-	-	+	+	+	+	+	+	-
6	-	-	+	+	+	+	-	-	-
7	-	-	+	+	+	+	+	+	-
8	-	-	+	+	+	+	+	+	-
9	-	-	+	+	+	+	+	+	+
10	-	-	+	+	+	+	+	+	+
11	-	-	+	+	+	+	+	+	-
12	-	-	+	+	+	+	+	+	+
13	-	-	+	+	+	+	+	+	-
14	-	-	+	+	+	+	+	+	+
15	-	-	+	+	+	+	+	-	-
16	-	-	-	-	-	+	-	-	-
17	-	-	+	+	+	+	+	+	+
18	-	-	+	+	+	+	+	+	-
19	-	-	+	+	+	+	+	+	+
20	-	-	+	+	+	+	+	+	+
21	-	-	+	+	+	+	+	+	-
22	-	-	+	+	+	+	+	+	+
23	-	-	+	+	+	+	-	-	-
24	-	-	-	-	+	+	-	-	-
25	-	-	+	+	+	+	+	+	+
26	-	-	-	-	+	+	-	-	-
27	-	-	+	+	+	+	+	+	+
28	-	-	-	-	-	+	-	-	-
29	-	-	-	-	+	+	-	-	-
30	-	-	+	+	+	+	+	+	+
31	-	-	-	+	+	+	-	-	-
32	-	-	+	+	+	+	+	+	+
33	-	-	-	-	+	+	-	-	-
34	-	-	-	+	+	+	+	+	+
35	-	-	+	+	+	+	+	+	-

+: Growth.
-: No Growth.

Table 4. Effects of Various Sodium Chloride Concentrations on the Growth of the Isolates.

	Sodium Chloride Concentration						
Strain	0.0%	0.2%	0.4%	0.6%	0.8%	1.0%	3.0%
1	++	++	++	++	++	++	-
2	++	++	++	++	++	++	-
3	++	++	++	++	++	++	-
4	++	++	++	++	++	++	-
5	++	++	++	++	++	++	-
6	++	+	-	-	-	-	-
7	++	++	++	++	++	++	-
8	++	++	++	++	++	++	-
9	++	++	++	++	++	++	-
10	++	++	++	++	++	++	-
11	++	++	++	++	++	++	-
12	++	++	++	++	++	++	-
13	++	++	++	++	++	++	-
14	++	++	++	++	++	++	-
15	++	++	++	++	++	++	-
16	++	++	++	-	-	-	-
17	++	++	++	++	++	++	-
18	++	++	++	++	++	++	-
19	++	++	++	++	++	++	-
20	++	++	++	++	++	++	-
21	++	++	++	++	++	++	-
22	++	++	++	++	++	++	-
23	++	++	++	+	-	-	-
24	++	+	+	-	-	-	-
25	++	++	++	++	++	++	-
26	++	++	++	-	-	-	-
27	++	++	++	++	++	++	-
28	++	-	-	-	-	-	-
29	++	++	-	-	-	-	-
30	++	++	++	++	++	++	-
31	++	++	++	-	-	-	-
32	++	++	++	++	++	++	-
33	++	-	-	-	-	-	-
34	++	++	++	++	++	++	-
35	++	++	++	++	++	++	-

++: Growth.
+: Poor Growth.
-: No Growth.

Effects of Anaerobic Conditions on Growth

A procedure described by Anderson and Ordal (4) was used to determine if the isolates were capable of growth under anaerobic conditions. In preparing the medium, it was noted that the pH changed from 7.2 to approximately 8.0 after the addition of the filter sterilized sodium bicarbonate. Consequently both phosphate buffered and unbuffered anaerobic media were tested. The results of these studies are found in Table 5. At pH 8.0, five of the 35 isolates grew under anaerobic conditions. In the buffered medium, containing 0.05M phosphate, a pH of 7.2 was maintained after the addition of the bicarbonate. Twenty-five of the isolates were able to grow anaerobically in the phosphate buffered medium. If the bicarbonate was omitted from the unbuffered medium, 33 of the 35 isolates were able to grow. Those isolates which grew in the phosphate buffered medium anaerobically also grew in this medium aerobically as did four additional isolates. All of the isolates grew aerobically if the bicarbonate is omitted from the medium. The findings from this study indicate that all but two of the isolates are facultative anaerobes since they grew both in the presence and absence of oxygen. Furthermore, CO_2 was not required for anaerobic growth as has been previously reported for Cytophaga succinicans (4).

Table 5. The Effect of Anaerobic Conditions on the Growth of the Isolates.

	Anaerobic Growth*			Aerobic Growth*	
Strain	Unbuffered (pH 8)	Buffered (pH 7.2)	Minus $NaHCO_3$ (pH 7.2)	Buffered (pH 7.2)	Minus $NaHCO_3$ (pH 7.2)
1	-	+	+	+	+
2	±	+	+	+	+
3	-	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	-	-	-	-	+
7	-	+	+	+	+
8	-	+	+	+	+
9	-	+	+	+	+
10	-	+	+	+	+
11	-	+	+	+	+
12	-	+	+	+	+
13	-	+	+	+	+
14	-	+	+	+	+
15	-	±	+	+	+
16	-	-	-	-	+
17	+	+	+	+	+
18	-	±	+	+	+
19	-	+	+	+	+
20	-	±	+	+	+
21	-	+	+	+	+
22	-	+	+	+	+
23	-	±	+	+	+
24	-	+	+	+	+
25	-	±	+	+	+
26	-	+	+	±	+
27	-	+	+	+	+
28	-	±	+	-	+
29	-	-	+	-	+
30	-	+	+	+	+
31	+	+	+	+	+
32	-	+	+	+	+
33	-	±	+	-	+
34	+	+	+	+	+
35	-	+	+	+	+

+: Growth.

-: No growth.

±: Growth questionable.

*: The basal medium consisted of that described by Anderson and Ordal (4).

Sensitivity of the Isolates to Selected Antibiotics

The effect of antibiotics on the isolates is presented in Table 6. The data indicate that all 35 isolates were sensitive to both tetracycline (5 μg) and streptomycin (10 μg). Thirty-one of the cultures were sensitive to novobiocin (5 μg) while 30 were sensitive to erythromycin (2 μg). The isolates were shown to be least sensitive to neomycin (5 μg) which inhibited the growth of only one culture, and polymyxin B (50 units) which inhibited the growth of four isolates. Penicillin G (2 units) and bacitracin (2 units) were both found to be effective against 10 of the 35 isolates while gantrisin (50 μg) inhibited the growth of 11 cultures.

Heat Resistance

The ability of the isolates to survive exposures to temperatures of 37C, 50C, 55C and 70C for varying lengths of time is summarized in Table 7. When vegetative cells from two day old cultures were exposed to a temperature of 37C for 5 minutes, only two isolates were killed. Following exposure of the cells to a temperature of 55C for 5 minutes, 28 of the isolates remained viable. Twenty-seven of the isolates survived a 15 minute exposure at 55C.

Cells from 30 day old cultures were held at 50C for 5 minutes and 28 of the 35 isolates were found to remain viable. The same

Table 6. Sensitivity of Isolates to Selected Antibiotics.

	Antibiotic								
Strain	tetracycline 5 ug	streptomycin 10 ug	penicillin G 2 units	bacitracin 2 units	novobiocin 5 ug	erythromycin 2 ug	gantrisin 50 ug	neomycin 5 ug	polymyxin B 50 units
1	+	+	-	-	+	+	-	-	-
2	+	+	-	-	+	+	-	-	-
3	+	+	-	-	+	+	-	-	-
4	+	+	-	-	+	+	+	-	-
5	+	+	-	+	+	+	-	-	-
6	+	+	-	+	-	+	-	-	+
7	+	+	-	+	+	+	-	-	-
8	+	+	+	-	+	-	-	-	-
9	+	+	-	-	+	-	-	-	-
10	+	+	-	-	+	+	-	-	-
11	+	+	+	-	+	+	-	-	-
12	+	+	-	-	-	+	-	-	-
13	+	+	-	-	+	-	-	-	-
14	+	+	-	-	+	+	+	-	-
15	+	+	-	-	+	-	-	-	-
16	+	+	+	+	+	+	+	-	+
17	+	+	-	-	+	+	-	-	-
18	+	+	-	+	+	+	-	-	-
19	+	+	-	-	-	+	-	-	-
20	+	+	-	+	+	+	+	-	+
21	+	+	-	-	+	+	-	-	-
22	+	+	-	-	+	+	-	-	-
23	+	+	-	-	+	+	+	-	-
24	+	+	+	-	+	+	-	-	-
25	+	+	+	-	+	+	-	-	-
26	+	+	+	-	+	+	+	-	-
27	+	+	-	-	+	+	-	-	-
28	+	+	+	+	+	+	+	+	+
29	+	+	+	+	+	+	+	-	-
30	+	+	-	-	-	+	+	-	-
31	+	+	-	-	+	-	-	-	-
32	+	+	-	-	+	+	+	-	-
33	+	+	+	+	+	+	+	-	-
34	+	+	+	-	+	+	-	-	-
35	+	+	-	+	+	+	-	-	-

+: Sensitive.

-: Resistant.

Table 7. Heat Resistance of Young and Old Cells of the Isolates.

	2 Day Cells				30 Day Cells			
	37C		55C		50C		70C	
Strain	5'	15'	5'	15'	5'	15'	5'	15'
1	+	+	+	+	+	+	-	-
2	+	+	+	+	+	+	-	-
3	+	+	+	+	+	+	-	-
4	+	+	+	+	+	+	-	-
5	+	+	+	+	+	+	-	-
6	+	+	+	+	-	-	-	-
7	+	+	+	+	+	+	-	-
8	+	+	+	+	+	+	-	-
9	+	+	+	+	+	+	-	-
10	+	+	+	+	+	+	-	-
11	+	+	+	-	+	+	-	-
12	+	+	+	+	+	+	-	-
13	+	+	+	-	+	+	-	-
14	+	+	+	+	+	+	-	-
15	+	+	+	+	+	+	-	-
16	-	-	-	-	-	-	-	-
17	+	+	+	+	+	+	-	-
18	+	+	+	-	+	+	-	-
19	+	+	±	+	+	+	-	-
20	+	+	+	+	+	+	-	-
21	+	+	+	+	+	+	-	-
22	+	+	+	+	+	+	-	-
23	+	+	+	+	+	-	-	-
24	+	+	+	+	-	-	-	-
25	+	+	+	+	+	+	-	-
26	+	+	+	-	+	+	-	-
27	+	+	+	+	+	+	-	-
28	-	-	-	-	-	-	-	-
29	+	+	±	+	-	-	-	-
30	+	+	+	+	+	+	-	-
31	+	+	±	+	+	+	-	-
32	+	+	+	+	+	+	-	-
33	+	+	-	-	-	-	-	-
34	+	+	-	-	+	+	-	-
35	+	+	+	+	+	+	-	-

+: Growth.
-: No Growth.
±: Growth Questioned.

results were obtained when these cells were held at 50C for 15 minutes. Exposure of 30 day old cells to 70C for 5 minutes, however, resulted in the complete inhibition of growth for all cultures.

Biochemical Characteristics

Carbohydrate Utilization

Data presented in Table 8 indicate the carbohydrates utilized by the isolates as determined by the modified Hugh-Leifson method (68). It can be seen that the majority of the organisms are capable of oxidizing carbohydrates. Of the 35 cultures examined, 30 oxidize glucose. Nearly all of these 30 also oxidize galactose, maltose and cellobiose. Lactose was oxidized by 17 of the isolates and sucrose by only 13. Only one isolate, strain 12, was able to oxidize mannitol.

Fermentative organisms were also detected among the isolates. As shown in Table 8, glucose, maltose and galactose were fermented by five strains. In addition, four strains fermented sucrose, lactose and cellobiose. These fermentative organisms are of interest since there have been very few reports of fermentative myxobacteria isolated from aquatic environs.

When the isolates were tested to determine CO_2 dependency of glucose fermentation four of the 35 grew, producing acid indicating anaerobic fermentation of this substrate. Of these, only one, strain

Table 8. Ability of the Isolates to Ferment or Oxidize Various Carbohydrates.

Strain	Glucose	Galactose	Maltose	Cellobiose	Sucrose	Lactose	Mannitol	CO_2-Glucose*
1	O	O	O	O	N	O	N	-
2	O	O	O	O	N	O	N	-
3	O	O	O	O	O	O	N	-
4	O	O	O	O	N	-	N	-
5	O	O	O	O	N	-	N	-
6	O	O	O	O	O	O	N	-
7	O	O	O	O	N	O	N	-
8	O	O	O	O	N	O	N	-
9	O	O	O	O	O	O	N	-
10	O	O	O	O	N	N	N	-
11	O	O	O	O	N	-	N	-
12	O	O	O	O	O	-	O	-
13	O	O	-	O	N	N	N	-
14	O	O	O	O	N	N	N	-
15	O	O	O	O	O	O	N	-
16	-	-	X	N	-	-	N	-
17	O	O	O	O	-	N	N	-
18	O	O	O	O	N	O	N	-
19	O	O	-	O	N	O	N	-
20	N	-	N	N	N	-	N	-
21	O	O	O	O	O	O	N	-
22	O	-	O	O	N	N	N	-
23	F	F	F	F	F	F	N	F
24	F	F	F	F	F	-	N	-
25	O	O	O	O	O	O	N	-
26	F	F	F	F	F	F	N	-
27	O	F	O	O	O	N	N	-
28	-	N	N	N	N	N	X	-
29	-	N	O	-	N	N	N	-
30	O	-	O	N	N	N	N	-
31	F	F	F	F	F	F	N	F
32	O	O	O	O	O	-	N	-
33	N	N	N	N	N	N	N	-
34	F	F	F	-	N	F	N	F
35	O	O	O	O	N	O	N	F

O: Oxidizer; acid produce aerobically.

F: Fermenter; acid produced aerobically and anaerobically.

N: Nonoxidizer-nonfermenter; alkaline reaction aerobically.

-: Growth; no acid or alkaline reactions.

X: No results obtained.

*: CO_2-dependent fermentation of glucose; unbuffered medium, pH 8; Anderson and Ordal (4).

35, did not ferment glucose anaerobically when using the Hugh-Leifson method (68) in the absence of CO_2. Strain 35 apparently requires CO_2 for anaerobic glucose fermentation as has been reported for Cytophaga succinicans (4).

Degradation of Macromolecules

The ability of the different organisms to degrade macromolecules was tested and the results are presented in Table 9. Most of the test organisms exhibited a high degree of hydrolytic activity. All but two of the isolates, strains 20 and 28, were able to hydrolyze starch. Casein and gelatin were hydrolyzed by all except culture 20.

Lipolytic action was determined by examining tributyrin plates for clear zones around colony growth. All of the isolates were able to hydrolyze this substrate.

The ability of the test isolates to hydrolyze aesculin was determined by observing plates for the formation of a black precipitate around the colonies in the presence of ferric ammonium citrate on aesculin plates. Thirty-four of the 35 cultures were able to hydrolyze aesculin. Only strain 20 failed to hydrolyze this compound.

Cellulose degradation was tested initially using a mineral basal medium containing cellulose powder and none of the organisms were found to utilize this compound. In subsequent tests carboxymethyl cellulose plates were used. Twenty-five of the cultures were able to

Table 9. Ability of the Isolates to Degrade Selected Macromolecules.

Strain	Starch	Casein	Gelatin	Tributyrin	Aesculin	Cellulose Powder	CMC*	Chitin
1	+	+	+	+	+	-	+	-
2	+	+	+	+	+	-	+	-
3	+	+	+	+	+	-	+	-
4	+	+	+	+	+	-	+	-
5	+	+	+	+	+	-	+	-
6	+	+	+	+	+	-	-	-
7	+	+	+	+	+	-	+	-
8	+	+	+	+	+	-	+	+
9	+	+	+	+	+	-	+	-
10	+	+	+	+	+	-	+	+
11	+	+	+	+	+	-	+	+
12	+	+	+	+	+	-	+	-
13	+	+	+	+	+	-	+	+
14	+	+	+	+	+	-	+	-
15	+	+	+	+	+	-	+	-
16	+	+	+	+	+	-	-	-
17	+	+	+	+	+	-	+	-
18	+	+	+	+	+	-	+	-
19	+	+	+	+	+	-	-	-
20	-	-	-	+	-	-	-	-
21	+	+	+	+	+	-	-	-
22	+	+	+	+	+	-	-	-
23	+	+	+	+	+	-	-	-
24	+	+	+	+	+	-	+	-
25	+	+	+	+	+	-	+	-
26	+	+	+	+	+	-	+	-
27	+	+	+	+	+	-	+	-
28	-	+	+	+	+	-	-	-
29	+	+	+	+	+	-	+	+
30	+	+	+	+	+	-	-	-
31	+	+	+	+	+	-	+	-
32	+	+	+	+	+	-	+	+
33	+	+	+	+	+	-	+	-
34	+	+	+	+	+	-	-	-
35	+	+	+	+	+	-	+	-

+: Macromolecule hydrolyzed.
-: Growth, but no hydrolysis.
*: Carboxymethyl cellulose.

utilize cellulose in this form.

While all 35 isolates were able to grow on the chitin test medium, only six were able to degrade this complex macromolecule as indicated by clear zones around colony growth.

Miscellaneous Physiological Tests

A number of additional physiological tests were carried out to further characterize the isolates. The results of these studies are presented in Table 10.

The majority of the cultures were found to reduce nitrate aerobically. Anaerobically, 17 of the 35 isolates were able to reduce nitrate indicating that these organisms could use NO_3 as a terminal hydrogen acceptor. Two of the isolates, strains 19 and 20, were found to produce gas both aerobically and anaerobically in nitrate broth. These same two cultures were subsequently found to be denitrifiers when tested in the modified denitrifing medium (14).

Only four of the isolates produce H_2S from 0.4% tryptone broth as determined by the use of lead acetate test strips.

Physiological studies including urea breakdown, decarboxylation of lysine, and ammonia production from arginine were also conducted. Isolates were never capable of urease formation nor were they able to convert lysine to cadaverine. However, 26 of the 35 were able to deaminate arginine thereby liberating ammonia as detected by the use

Table 10. Miscellaneous Physiological Reactions of the Isolates.

Strain	H_2S	NO_3 Reduction Aerobic	NO_3 Reduction Anaerobic	Denitrification	Urease	Lysine Decarboxylase	Arginine Deaminase
1	-	-	-	-	-	-	+
2	-	+	+	-	-	-	+
3	S	+	-	-	-	-	+
4	S	+	+	-	-	-	+
5	-	-	-	-	-	-	+
6	-	-	-	-	-	-	-
7	-	+	-	-	-	-	+
8	-	+	+	-	-	-	+
9	-	+	-	-	-	-	+
10	-	+	+	-	-	-	+
11	-	-	-	-	-	-	+
12	-	+	+	-	-	-	+
13	-	+	+	-	-	-	+
14	-	+	+	-	-	-	+
15	-	+	+	-	-	-	+
16	-	-	-	-	-	-	-
17	-	+	+	-	-	-	+
18	-	+	-	-	-	-	+
19	S	+o	+o	+	-	-	+
20	-	+o	+o	+	-	-	-
21	-	-	-	-	-	-	+
22	-	-	-	-	-	-	+
23	-	+	+	-	-	-	-
24	-	+	+	-	-	-	-
25	-	-	-	-	-	-	+
26	-	-	-	-	-	-	+
27	-	+	+	-	-	-	+
28	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-
30	S	-	-	-	-	-	+
31	-	+	+	-	-	-	+
32	-	+	-	-	-	-	+
33	-	-	-	-	-	-	-
34	-	+	+	-	-	-	-
35	-	+	+	-	-	-	+

+: NO_3 reduced; Denitrification takes place; Urease, lysine decarboxylase and arginine deaminase produced.

-: Growth present but negative test.

o: Gas produced.

S: H_2S produced.

Table 10. (Continued)

Strain	Tyrosine	Litmus Milk	Indol	Methyl Red	VP*	Catalase	Cytochrome Oxidase
1	G	L; p	-	-	-	+	-
2	G	L; p	-	-	-	+	-
3	G	L; p	-	-	-	+	-
4	G	L; p	-	-	-	+	-
5	G	L; p	-	-	-	+	-
6	G	L; p	-	-	-	+	-
7	T	L; p	-	-	-	+	-
8	G	L; p	-	-	-	+	-
9	G	L; p	-	-	-	+	-
10	G	L; p	-	-	-	+	-
11	P	L; p	-	-	-	+	-
12	G	L; p	-	-	-	+	-
13	G	L; p	-	-	-	+	-
14	G	L; p	-	-	-	+	-
15	G	L; p	-	-	-	+	-
16	T; P	L; p	-	-	-	+	-
17	G	L; p	-	-	-	+	-
18	G	L; p	-	-	-	+	-
19	T; P	L; p	-	-	-	+	-
20	T	L; p	-	-	-	+	-
21	T; P	L; p	-	-	-	+	-
22	T; P	L	-	-	-	+	-
23	G	L; p	-	-	-	+	-
24	G	NC	-	-	-	+	-
25	P'	L; p	-	-	-	+	-
26	T	L	-	-	-	+	-
27	P	L	-	-	-	+	-
28	T	L; p	-	-	-	+	-
29	G	L; p	-	-	-	+	-
30	G	L; p	-	-	-	+	-
31	G	L; p	-	-	-	+	-
32	G	L; p	-	-	-	+	-
33	G	L; p	-	-	-	+	-
34	T; P	L	-	-	-	+	-
35	G	L; p	-	-	-	+	-

+: Indol, methyl red, acetylmethyl carbonyl, catalase and cytochrome oxidase produced.
-: Growth present but negative results.
G: Growth on tyrosine; no hydrolysis or pigment production.
T: Tyrosine hydrolyzed.
P: Pigment produced from tyrosine.
L: Litmus milk reduced.
p: Litmus milk peptonized.
NC: No change in litmus milk.
*: Voges-Proskauer test for acetylmethyl carbonyl.

of Nessler's reagent.

Nine of the isolates decomposed tyrosine as detected by the disappearance of tyrosine from around colony growth. Of the nine able to decompose this compound, cultures 16, 19, 21, 22 and 34 produced a diffusible melanin-like pigment from the tyrosine while cultures 7, 20, 26 and 28 hydrolyzed the substrate without pigment production. Three isolates, cultures 11, 25 and 27, produced this pigment without hydrolysis of the tyrosine.

With the exception of culture 24 all of the isolates were able to grow in litmus milk. All of the cultures that were able to grow also reduced litmus milk. Cultures 22, 26, 27 and 34, which were able to hydrolyze casein failed to exhibit proteolytic activity when cultured in litmus milk. Culture 20 which was not able to hydrolyze casein, however, was found to be proteolytic in this medium. The reason for this discrepancy is not clear. In addition to culture 20, 29 other isolates also exhibited proteolytic activity in litmus milk.

None of the 35 isolates gave positive indol, methyl red or Voges-Proskauer (acetyl methyl carbinol) reactions. All were able to grow in the test media employed.

All 35 isolates were catalase positive. However, cultures 16 and 26 were weakly positive and without careful examination it would be possible to mistake this for a negative test.

The isolates were tested for cytochrome oxidase. This enzyme

is required for the oxidation of dimethyl-phenylene-diamine in the presence of molecular oxygen and cytochrome c. All 35 isolates gave a negative indolphenol blue color when alpha-naphthol was added to the incubated medium indicating cytochrome oxidase was not present.

Nutritional Studies

Stanier's (75) mineral basal medium containing soluble potato starch as the carbon source was used to study the nitrogen requirements of the organisms. The nitrogen sources tested included casein hydrolysate, $(NH_4)_2SO_4$, KNO_3 and KNO_2. As shown in Table 11, all of the isolates were able to grow when either casein hydrolysate or KNO_3 were used as the sole nitrogen source although a few strains grew rather poorly when KNO_3 was employed. When $(NH_4)_2SO_4$ was used, 28 of the isolates were able to initiate growth. Fifteen of the cultures were able to grow when KNO_2 was used as the sole nitrogen source and an additional six cultures showed scanty growth. From these findings it is apparent that an organic nitrogen source serves as the most widely utilized source for these cultures although the majority also have the capacity to use inorganic forms of nitrogen.

An attempt was made to determine if the isolates could fix free atmospheric nitrogen. The isolates were inoculated into the mineral basal medium containing soluble potato starch in the absence of an added nitrogen source. Preliminary results showed that most of the

Table 11. The Ability of the Isolates to Utilize Various Compounds as Sole Sources of Nitrogen.

Strain	Casein Hydrolysate	$(NH_4)_2SO_4$	KNO_3	KNO_2
1	+	+	+	±
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	-	+	+
7	+	+	+	±
8	+	±	±	+
9	+	+	+	+
10	+	+	+	+
11	+	+	+	-
12	+	+	+	+
13	+	+	+	+
14	+	+	+	-
15	+	+	+	±
16	+	-	±	±
17	+	+	+	+
18	+	+	+	±
19	+	+	+	-
20	+	-	±	-
21	+	+	+	+
22	+	+	+	+
23	+	+	+	-
24	+	-	+	±
25	+	+	+	-
26	+	-	+	-
27	+	+	+	-
28	+	-	±	-
29	±	-	±	-
30	+	+	+	+
31	+	-	±	+
32	+	+	+	+
33	+	+	+	-
34	+	+	+	-
35	+	+	+	-

+: Growth.
-: No Growth.
±: Poor Growth.

isolates were able to grow in this medium suggesting that atmospheric nitrogen was being used by these cultures. To test this possibility further inoculated tubes were placed in a gasing jar, flushed with pure oxygen and allowed to incubate for one week. At the end of this period 29 of the isolates showed evidence of growth. One loop transfers were then made into a fresh medium and the procedure repeated. After the second incubation period only 23 were found to grow. These results suggested that the organisms were using a contaminating source of nitrogen in the medium rather than fixing atmospheric nitrogen.

Uninoculated test medium was then checked for the presence of NH_3, NO_3 or NO_2 by using Nessler's reagent, sulfanilic acid and dimethyl-alpha-naphthylamine, and Trommsdorf reagents. Trace amounts of NO_2 were shown to be present in the medium. Since several isolates are able to utilize NO_2 (Table 11) further purification of the reagents must be made before N_2 utilization can be determined.

The same mineral basal medium containing KNO_3 as the nitrogen source was used in determining whether the isolates could use glucose, cellobiose, citrate or sodium bicarbonate as sole sources of carbon (Table 12). Of the 13 isolates able to utilize cellobiose as their sole carbon source, 10 were also able to use glucose. Glucose was also able to support the growth of two additional isolates. None of the cultures were able to grow when sodium bicarbonate was used

Table 12. The Ability of the Isolates to Utilize Various Compounds as Sole Sources of Carbon.

Strain	Glu-cose	Cello-biose	Cit-rate	$NaHCO_3$	Argi-nine	Cyste-ine	Ala-nine	Gly-cine	Gluta-mine	Pro-line
1	-	±	-	-	-	-	-	-	-	-
2	±	±	-	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-	-
4	+	+	-	-	-	-	-	-	-	-
5	-	±	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-
7	+	+	-	-	-	-	-	-	-	-
8	+	+	-	-	-	-	-	-	-	-
9	+	+	-	-	-	-	-	-	-	-
10	-	±	-	-	-	-	-	-	-	-
11	+	+	-	-	-	-	-	-	-	-
12	-	±	-	-	-	-	-	-	-	-
13	-	±	-	-	-	-	-	-	-	-
14	+	+	+	-	-	-	-	-	-	-
15	+	+	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-
17	+	+	-	-	-	-	-	-	-	-
18	-	±	-	-	-	-	-	-	-	-
19	±	-	-	-	-	-	-	-	-	-
20	+	-	-	-	-	-	-	-	-	-
21	-	+	-	-	-	-	-	-	-	-
22	+	-	+	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-
24	+	+	-	-	-	-	-	-	-	-
25	-	±	-	-	-	-	-	-	-	-
26	-	-	+	-	-	-	-	-	-	-
27	±	+	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-
30	±	-	-	-	-	-	-	-	-	-
31	-	+	-	-	-	-	-	-	-	-
32	-	±	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-
34	±	-	-	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-	-	-	-

+: Growth.
-: No Growth.
±: Growth Questioned.

as the sole carbon source.

When citrate was tested, three of the 35 isolates were found capable of utilizing this substrate as their sole carbon source. One of the three was also able to utilize both glucose and cellobiose; one, only glucose; and, the third, only the citrate.

The ability of the isolates to utilize the amino acids arginine, alanine, cysteine, glycine, proline or glutamic acid as a sole source of carbon was also tested (Table 12). These amino acids were unable to support the growth of the isolates, and thus could not be used to satisfy the sole carbon requirement of this group of aquatic myxobacteria.

Lytic Action

Numerous reports (27, 34, 39, 47, 53, 62, 63, 65, 70, 83, 85, 90) have indicated that some myxobacteria are able to lyse cells of certain bacteria, fungi, blue-green algae and nematodes. The fresh water isolates in this investigation were examined for their ability to lyse dead cell preparations of gram negative and gram positive bacteria, yeast, algae and a protozoan. A list of the cultures used and the results obtained in this study are summarized in Table 13.

All of the isolates were able to lyse dead cells of Aerobacter aerogenes. The majority of the isolates also lysed cells of Corynebacterium hofmanii, Sarcina ureae, Pseudomonas aeruginosa,

Table 13. The Ability of the Isolates to Lyse Dead Cells of Selected Microorganisms.

Strain	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	+	-	+	+	+	+	+	+	±	+	+	+	-	-	+	-	+
2	-	-	-	+	+	-	-	±	-	+	+	-	-	-	±	-	+
3	±	-	+	+	+	+	+	+	±	+	+	±	-	-	+	-	+
4	+	-	+	+	+	+	+	+	±	+	+	±	-	-	+	±	+
5	±	-	+	+	+	+	+	+	±	+	+	-	-	-	+	-	+
6	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	+
7	-	-	+	+	+	+	+	+	±	+	+	±	-	-	+	-	+
8	-	-	+	+	+	+	+	±	-	+	+	-	-	-	+	-	+
9	±	-	+	±	+	+	+	+	±	+	+	-	-	-	+	-	+
10	±	-	+	+	+	+	+	+	-	+	+	-	-	-	+	-	+
11	±	-	+	+	+	+	+	+	-	+	+	-	-	-	+	-	+
12	±	-	+	+	+	+	+	+	±	+	+	-	±	-	+	-	+
13	±	-	+	+	+	+	+	+	±	+	+	-	-	-	+	-	+
14	±	-	+	+	+	+	+	+	±	+	+	±	-	-	+	±	+
15	-	-	+	+	+	±	+	+	-	+	+	-	-	-	+	-	+
16	±	-	+	+	+	+	+	+	±	+	+	±	-	-	+	-	+
17	-	-	+	+	+	+	+	+	-	+	+	-	-	-	+	-	+
18	-	-	+	+	+	+	+	+	-	+	+	-	±	-	+	-	+
19	-	-	+	+	±	-	+	+	-	+	+	-	-	-	+	-	+
20	-	-	-	-	-	-	-	-	-	+	-	±	-	-	-	-	-
21	±	-	+	+	±	+	+	+	-	+	+	-	±	-	+	±	+
22	-	-	+	+	±	+	+	+	-	+	+	-	±	-	+	-	+
23	-	-	+	+	+	-	+	+	-	+	+	-	-	-	+	-	+
24	±	-	+	+	±	+	+	+	-	+	+	-	-	-	+	-	+
25	-	-	+	±	±	-	+	±	-	+	+	-	±	-	+	-	±
26	-	-	+	+	+	+	+	+	-	+	+	±	-	-	+	-	+
27	-	-	+	+	±	+	+	+	-	+	+	±	-	-	+	-	+
28	±	-	+	+	+	+	+	+	-	+	+	-	±	-	+	-	+
29	±	-	+	+	+	+	+	+	-	+	+	±	±	-	+	+	+
30	±	-	+	+	+	+	+	±	-	+	+	-	-	-	+	-	±
31	-	-	-	±	±	+	+	-	-	+	+	-	-	-	-	-	-
32	-	-	+	+	+	+	+	±	-	+	+	-	±	-	+	-	+
33	±	-	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+
34	-	-	+	+	+	+	+	+	-	+	+	-	-	-	+	-	±
35	-	-	+	+	+	+	+	+	-	+	+	-	-	-	+	-	+

+: Dead Cells Lysed.

-: Growth; Dead Cells Not Lysed.

±: Lysis Questionable.

A: Anabeana sp.
B: Scenedesmus obliqus strain D_3
C: Arthrobacter sp.
D: Corynebacterium hofmanii
E: Alcaligenes viscolactis
F: Sarcina ureae
G: Pseudomonas aeruginosa
H: Bacillus megatherium
I: Bacillus subtilis
J: Aerobacter aerogenes
K: Escherichia coli
L: Streptococcus faecalis
M: Staphylococcus aureus
N: Mycobacterium smegmatis
O: Proteus vulgaris
P: Englena gracilis
Q: Saccharomyces cerevisiae

Bacillus megatherium, Escherichia coli, Proteus vulgaris, Saccharomyces cerevisiae and Arthrobacter sp. The other organisms employed as substrates were lysed by one or more of the isolates although in some cases lysis was questionable. There appears to be no preference for gram positive or negative organisms. It was also interesting to note that a few of the isolates appeared to be able to lyse cells of the green algae, Scenedesmus obliqus strain D_3, and the blue-green algae, Anabeana sp., as well as the protozoan, Euglena gracilis.

DNA: Tm and Base Ratio Values

DNA was isolated from cultures 4, 8, 20, 25, 33 and 34 and thermal melting point (Tm) values were determined for each. Culture 34 was chosen as one of the six isolates for this study since it was a known species of the genus Cytophaga and had a published G+C value of 38% (44). It was also representative of typical yellow colored cytophagas. Cultures 8, 25 and 33 were chosen because of their different pigmentation: culture 8 was yellow-orange, culture 25 produced a brown diffusible pigment, and culture 33 was a pink species. Cultures 4 and 20 were chosen because they had been tentatively assigned to the genus Sporocytophaga and they were pigmented yellow and white respectively. Calf thymus DNA, which has a published Tm value of 87C, was used as the control for each Tm determination.

The Tm values obtained from duplicate thermal denaturation curves are presented in Table 14. The % GC values obtained ranged from 34.88 to 38.54% for cultures 4, 8, 25, 33 and 34. The % GC value calculated for culture 20 was 52.44%.

Table 14. Tm and Base Ratios of DNA of Selected Isolates.

Strain	Tm	Average Tm*	Average % G+C**
4	83.10C 84.10C	83.60C	34.88
8	84.50C 84.95C	84.72C	37.61
20	91.46C 90.15C	90.80C	52.44
25	84.55C 84.20C	84.38C	36.78
33	83.20C 84.25C	83.72C	35.17
34	84.95C 85.25C	85.10C	38.54

*Average of two determinations.

**Calculated on the basis of the average Tm using the formula of Marmur and Doty (57): Tm = 69.3 + 0.41 (G+C).

Pigment Analysis

Carotenoids are widely distributed throughout the plant and animal kingdoms but are synthesized only by plants and microorganisms. Considerable information is available concerning the carotenoids of plants due the availability of large amounts of material for study. However, investigations of bacterial carotenoids has lagged because of the greater difficulties encountered in securing sufficient amounts of material with which to work. An attempt has been made to isolate and identify the carotenoid pigments produced by six different fresh water myxobacterial isolates in hopes of obtaining comparative biochemical information that would be of help in evaluating possible relationships occurring among these isolates and possibly aid in the classification of these same organisms. The isolates selected for this study are the same six that were used in the DNA analysis and were chosen for the same reasons as provided in that section.

Crude pigment extracts of cultures 4, 8, 20, 25, 33 and 34 were partitioned against petroleum ether and the pigments were separated into the epiphasic fraction. This fraction was evaporated to dryness, the residue resuspended in a small amount of diethyl ether and applied to a silica gel G column. Only one pigment band was obtained for each of the cultures 8, 20, 25 and 33 when ether was

used as the elutant. The epiphasic pigment extract of culture 4 yielded two separate pigment bands with the ether elutant. When culture 34 was eluted on the column, one pigment fraction came off with the diethyl ether and one with methanol elution.

The different pigment fractions obtained from elution of the silica gel G column were spotted onto thin layer plates and developed in a benzene:absolute methanol:glacial acetic acid (87:11:2; v:v:v) solvent. The plates were dried and the location of the pigment bands for each culture noted. Rf values for each band were calculated and are presented in Table 15 together with the spectral data for each of the isolated pigments.

Chromatographic development of the ether elutant of culture 34 indicated the presence of two pigment bands not completely separated from each other. Spectral analyses of these pigment bands indicated that the first band, the one which appeared to be common for most of the cultures examined, was spectrally similar to lutein while the second appeared most similar spectrally to the xanthophyll, alpha-carotene-5,6-epoxide. The only pigment found when the methanol fraction of culture 34 was chromatographed appeared to be similar to lutein. Earlier preliminary analysis had suggested the presence of lutein or isolutein in this culture.

The two pigments found when the ether fraction of culture 25 was chromatographed also appeared to be most similar spectrally to

Table 15. Absorption Maxima and Rf Units for the Different Pigments of Selected Isolates. Absorption spectra determined using a Beckman DK-2A Ratio Recording Spectrophotometer.

	Chloroform (mμ)			Carbon Disulfide (mμ)		
Strain 4:						
Ether fraction 1						
Pigment band #1	-	456	-	-	474	512
#2	-	-	-	-	473	510
#3	-	454	-	-	-	-
Ether fraction 2						
Pigment band #1	438.5	459	482	448	475.5	512
Strain 8:						
Ether fraction 1						
Pigment band #1	339	445.5	-	-	472	512
#2	-	446.8	-	459	471	-
#3	-	449	-	427.5	455	481
Strain 20:						
Ether fraction 1						
Pigment band #1	-	-	-	-	-	-
Strain 25:						
Ether fraction 1						
Pigment band #1	436	454.5	-	-	-	-
#2	438	454	476.5	-	469.5	504.5
Strain 33:						
Ether fraction 1						
Pigment band #1	-	481	507.1	-	507.5	-
#2	-	483.2	512	484.5	-	-
Strain 34:						
Ether fraction 1						
Pigment band #1	437.2	458.5	486	449	473.5	511
#2	-	454	482	-	-	-
Methanol fraction 1						
Pigment band #1	-	459	487	-	467	508

Table 15. (Continued)

	Hexane (mμ)			Rf
Strain 4:				
Ether fraction 1				
Pigment band #1	428	452	481	0.579
#2	-	453	-	0.636
#3	427	-	-	0.669
Ether fraction 2				
Pigment band #1	420.5	445.5	476	0.563
Strain 8:				
Ether fraction 1				
Pigment band #1	423	440	458	0.566
#2	424	443	460	0.661
#3	429	448	470	0.710
Strain 20:				
Ether fraction 1				
Pigment band #1	-	-	-	0.000
Strain 25:				
Ether fraction 1				
Pigment band #1	426	444	473.9	0.615
#2	422	440.5	474.5	0.763
Strain 33:				
Ether fraction 1				
Pigment band #1	-	471.0	497	0.575
#2	-	468.8	-	0.649
Strain 34:				
Ether fraction 1				
Pigment band #1	424.3	446.4	475.5	0.549
#2	-	-	-	0.625
Methanol fraction 1				
Pigment band #1	422	447	479	0.524

lutein and alpha-carotene-5, 6-epoxide. However, the second pigment band had an identical spectral pattern to helenien when the spectrum was recorded in n-hexane. Since spectral values for helenien are not available when run in carbon disulfide or chloroform it can only be speculated that this might indeed be the actual nature of this second pigment band.

Insufficient spectral data was obtained on culture 20 to make any comparison with known carotenoids. The pigment(s) from this culture remained at the point of origin on the thin layer plates and appeared light yellow in color. Absorption spectra provided insufficient data for the elucidation of this pigment fraction.

Culture 33 was conspicuously different from the other cultures. This culture possessed a predominate salmon-pink pigment which banded lower than those observed for the other cultures with the exception of culture 20. The pink pigment associated with this culture banded as one predominate band with a faint pigmented area above this band but not separated from it. Spectral data indicate a great similarity of these two pigment areas to the carotenoid rhodopin although there was also some similarity to lycopene. Earlier preliminary studies also suggested that the pigment fraction of this culture was similar spectrally to rhodopin.

When the first ether fraction of culture 4 was chromatographed, three bands were noted. The first of these bands seemed to be quite

similar to lutein when the spectral analysis was run in carbon disulfide and chloroform. Spectral evidence suggested that bands two and three were alike and that these appeared to be quite similar to alpha-carotene-5, 6-epoxide. When the second ether fraction was chromatographed only one pigment band was found and spectral analysis of this eluted pigment showed it to be almost identical to helenien in n-hexane but most similar to lutein when examined in chloroform or carbon disulfide. This particular pigment also banded slightly lower than the first pigment of the first ether fraction.

Three pigment areas were noted when the ether fraction of culture 8 was chromatographed. The top two areas were not clearly separated from each other. Spectral analysis resulted in peak values considerably different than those found for pigments of the other cultures, especially when determined in chloroform. The spectral data obtained for these pigments was not considered adequate enough to speculate upon their carotenoid character. On the basis of their thin layer chromatography patterns and partial spectral data from n-hexane it might be concluded that this culture possesses the characteristic yellow pigment found in the majority of the cultures examined which appears to be spectrally similar to the carotenoid lutein.

Bacteriophage

Lysed areas suggestive of bacteriophage activity were observed on various plates during the examination of cultural morphology of the isolates used in this investigation. A phage was subsequently isolated which infected and lysed not only the parent strain but nine other host strains. Up to this time published reports of phage for fresh water members of the genus Cytophaga have not been available.

Twenty-four hour enrichments of overnight broth cultures and water from Oak Creek were assayed using the agar layer method described by Adams (1) to determine plaque formation. Only cultures 2, 9, 17, 19, 21, 30, 32 and 34 showed evidence of plaques when assayed, and "crude" broth phage stocks were prepared for these cultures. These preparations were refrigerated at 8C without further attempting to purify or titer.

After storage for 12 months at 8C, the "crude" phage preparations were again plated out against their respective host strains. Only the phage preparation against culture 17 had remained viable during this period of time. The "crude" preparation was subsequently passed through a membrane filter to obtain a bacterial-free phage stock. This phage has been tentatively labeled ϕ-173-N for identification purposes. After filter sterilizing, ϕ-173-N was serially diluted and the titer determined. Titers as high as 10^8 per ml have been

obtained for this phage.

A host range study of the 35 isolates used in this investigation revealed isolates 1, 2, 10, 11, 12, 14, 15, 27 and 35 were "susceptible" to infection by ϕ-173-N. When this phage was assayed against these nine isolates, titers ranging from 10^2 to 10^4 were obtained (Table 16). Efficiency of plating values indicate that while genetically related in some way none of the host strains are identical to the parent strain. Also, three of the host strains appeared to be more genetically related to the parent strain than did the other six. The greatest efficiency of plating value was obtained when the homologous system of parent strain and ϕ-173-N was employed. These results are summarized in Table 16.

Table 16. Host Susceptibility to ϕ-173-N.

Host Strain	Plaque Count: Undiluted Phage	Plaque Count: Diluted Phage 1/10	Plaque Count: Diluted Phage 1/100	Titer (Phage/ml)	EOP**
1	> 300 > 300	7, 1	-	4.00×10^2	0.0023
2	> 300 > 300	316, 309	-	3.14×10^4	0.1825
10	> 300 > 300	340 -	-	3.40×10^4	0.1977
11	> 300 > 300	8, 7	-	7.50×10^2	0.0044
12	> 300 > 300	216, 198	-	2.04×10^4	0.1186
14	71 -	28 -	-	1.76×10^3	0.0102
15	> 300 > 300	82 -	-	8.20×10^3	0.0477
17*	> 300 > 300	> 300 > 300	173, 171	1.72×10^5	1.0000
27	> 300 > 300	70, 124	-	9.70×10^3	0.0564
35	239 183	-	-	2.11×10^3	0.0123

*: Parent strain against which ϕ-173-N was isolated.

**: Efficiency of plating; host strain titer/titer of parent strain.

DISCUSSION

Little information is available regarding the distribution, characteristics and activities of myxobacteria from fresh water environs. Consequently adequate identification of these organisms is often difficult to ascertain. This study was undertaken in an attempt to expand the present available knowledge concerning this group of organisms by providing taxonomic information on a number of fresh water myxobacterial isolates. A taxonomic scheme for the aquatic cytophagas will be proposed as a result of this investigation.

The 35 organisms studied were considered to be myxobacteria on the basis of their cellular morphology. The characteristic features noted during this investigation are similar to those reported by other workers (25, 75, 76, 86). Individual vegetative cells were found to be gram negative, unicellular rods with rounded ends which exhibited low refractility and a marked flexibility when observed under a phase contrast microscope. Typical creeping or gliding motility was easily demonstrated in young vegetative cells when phase contrast microscopy was employed.

Although several descriptions have been reported, no adequate explanation concerning the mode of action of myxobacterial locomotion has been proposed. Myxobacteria lack flagella, a characteristic of motile eubacteria. They also lack pseudopodia and cilia, modes of

locomotion characteristic of protozoa. Reports by Jahn and Bovee (42) and Dworkin (25) present a number of suggestions to explain myxobacterial motility. Some of these suggestions include directed slime production, cellular contraction, action of haustoria, or, the contraction of rhapidosomes. No attempt was made during the course of this investigation to determine the mechanism controlling this locomotion.

Young vegetative cells of the strains studied tended to be long and thin but upon aging became shorter and thicker and exhibited a reduced flexibility to the point of appearing as rigid as many true bacteria. With age, cells also tended to lose their gliding type of motility and involution forms appeared. Involution forms, also reported by other workers (11, 32, 68) were found to occur in rich media as well as in some Cytophaga broth cultures. As reported by Porter (68), once the cells reached this stage they would not revert to vegetative forms. Borg (11) suggests that lack of oxygen may be responsible for the development of involution forms.

A study of colony morphology revealed results similar to earlier reports in the literature (75, 78). The majority of the cultures observed produced typical thin, spreading, yellow to yellow-orange pigmented colonies. It was found that spreading was enhanced if the cultures were plated on a dilute medium such as 1/10 Cytophaga peptonized milk agar as compared to the richer Cytophaga or

peptonized milk agars. A similar effect was noted by Porter (68) and Stanier (78) when the agar concentration in the medium was varied. They found more compact colony formation at high concentrations of agar while spreading colonies occurred if lower concentrations were used.

The colony development of isolate 33 was found to be rather peculiar when compared to the other isolates. After three days of incubation, the pale pink colonies appeared to undergo a localized accumulation of cells in drop-like masses surrounding the major portion of the colony. These drop-like masses appeared at first to be similar to immature fruiting bodies but examination revealed normal vegetative cells. Microcyst formation was never observed in this culture. A similar type of colony morphology has been described for the marine-agar digesting bacterium, Cytophaga krzemienieskae (77).

According to the classification of the myxobacteria presented in Bergey's Manual of Determinative Bacteriology (12), two groups of non-fruiting myxobacteria are recognized. The genus Sporocytophaga is differentiated from the other members of the order Myxobacterales on the basis of microcyst formation in the absence of fruiting body structures. The members of the genus Cytophaga form neither fruiting bodies nor microcysts. In the present study, fruiting body structures were never observed in any of the isolates employed. However,

two of the cultures, strains 4 and 20, appeared capable of forming microcysts. Based on these observations, the majority of the organisms studied in this investigation are considered to be members of the genus Cytophaga. Isolates 4 and 20 are believed to be members of the genus Sporocytophaga.

Evidence of microcyst formation in culture 4 was particularly convincing. Examination of wet mounts revealed the appearance of refractile spherical structures which elongated into vegetative cells over a period of six hours. These structures fulfilled the majority of the criteria suggested by Dworkin (25) to be used as guide lines in establishing the presence of true microcyst structures. True microcysts are capable of germination, are resistant to physical or sonic destruction, are refractile under phase contrast microscopy, and the sequence of morphological events leading to their formation is clearly distinguishable from those characteristic of spheroplasts.

In culture 20 a major shortening and thickening of the cells occurred within 12 hours of incubation and the cells became quite coccoidal in shape. These coccoidal elements were found whenever this isolate was cultivated in a liquid medium. If these structures are observed over a period of six hours, cell elongation again occurs and it seems likely that they also are microcysts.

The results of the cultural and physiological tests performed on the fresh water isolates are summarized in Table 17. Also

included in this table are the number and the percentage of isolates giving positive results for each test. In general the isolates were aerobes which grew best at about 27C and at pH 7.2. The organisms did not require additional sodium chloride for growth and are considered to be non-halophilic mesophiles. While all of the isolates were found to grow at pH 7.2, it was also found that some were capable of initiating growth under more acid and/or more alkaline conditions. A greater tolerance to alkaline conditions was noted. Stanier (75) reports similar findings.

An attempt was made to determine growth under anaerobic conditions using a procedure described by Anderson and Ordal (4). Five of the 35 organisms were capable of anaerobic growth under the test conditions employed. It was noted however, that the pH of the growth medium changed from 7.2 to approximately 8.0 upon the addition of filter sterilized sodium bicarbonate. Anderson and Ordal (4) did not indicate whether or not the pH of the growth medium was readjusted to 7.2 after the addition of the bicarbonate and it seemed possible that the high pH could have influenced the results. To test this possibility a phosphate buffered medium was used in which the pH was maintained at 7.2. In this medium 25 of the isolates were able to grow anaerobically. When the bicarbonate was excluded from the unbuffered medium, 33 of the isolates grew anaerobically hence the majority of the organisms can be considered to be facultative anaerobes.

Furthermore none of the organisms appeared to require substrate amounts of CO_2 for anaerobic growth as reported for Cytophaga succinicans (4). In this regard it is interesting to note that the strain of Cytophaga succinicans studied in this investigation was capable of anaerobic growth in the absence of substrate amounts of CO_2. This strain cross reacts with antiserum prepared against Cytophaga succinicans and it is possible that this culture is a mutant which has lost the CO_2 requirement.

The lethal effect of heat on bacteria varies from species to species. The resistance of a given species is influenced by at least two factors: (a) the ability of the culture to form spores or microcysts, and (b) the conditions under which the organisms are cultivated. Spores are more resistant than vegetative cells to elevated temperatures and in some species can withstand temperatures of 100C for several hours. Vegetative cells of most bacteria, however, are killed when exposed to temperatures of 70C for 1 to 5 minutes. Some are killed when held at 54C for 10 minutes. To some extent, the time and temperature of incubation may influence the heat resistance of cells. Actively growing log phase cells are generally less resistant than cultures in the stationary phase of growth. Cultures grown at or above their optimal temperatures are more resistant than cultures grown at suboptimal temperatures.

The isolates examined in the present study appeared to be

moderately thermostable. Cultures of the majority of the organisms remained viable following exposure to temperatures as high as 55C for 15 minutes. Dworkin (25) has indicated that a number of reports exist in the literature regarding the greater heat resistance of microcyst structures over vegetative cells. Such reports indicate that microcysts can tolerate a temperature of 70C for 30 minutes and dry heat at 100C for 1 minute. Microcysts have also been held at 60C (dry heat) for 10 hours and still been capable of germination. Since none of the 30 day old cells were found to be viable after exposure to 70C for 5 minutes, it appears that microcyst formation does not readily occur among these isolates when cultivated in Cytophaga broth.

Little information is available concerning the antibiotic sensitivity of myxobacteria. Such information could be of use in ecological studies, for selective isolations, and, for understanding of myxobacterial structure and metabolism. A report by Henis and Kletter (38) indicates that sulphadiazine is an effective agent against myxobacteria and that these organisms are also inhibited by broad spectrum antibiotics such as aureomycin and chloramphenicol at normal concentrations. These authors also report myxobacteria are resistant to penicillin. In the present investigation, the isolates were found to be most sensitive to those antimicrobial agents which suppressed protein synthesis (tetracyclin, streptomycin, erythromycin and novobiocin) and least sensitive to those which disrupted

lipoprotein membranes (polymyxin B and neomycin). Less than half of the isolates appeared to be sensitive to antibiotics which suppressed cell wall synthesis (penicillin G, bacitracin and gantrisin).

Until recently, few reports of fermenting cytophages existed. In 1955 (5) the first report became available concerning the isolation of a Cytophaga strain capable of anaerobic growth at the expense of simple sugars such as glucose, sucrose and lactose. Since then other fermentative myxobacteria have been described (4, 88, 89). The isolation of Cytophaga succinicans by Anderson and Ordal (4) was of particular interest since this organism was isolated from fresh water rather than a marine environment and because it appeared to require CO_2 for anaerobic glucose fermentation.

Of the 35 isolates examined, 14% were able to ferment filter sterilized glucose, maltose and galactose. Eighty percent of those able to ferment glucose also fermented lactose, sucrose and cellobiose. Isolate 34, a culture of Cytophaga succinicans (RL-8) obtained from Dr. E. J. Ordal, was found to ferment glucose, lactose, maltose and galactose in the absence of CO_2 when using modified Hugh-Leifsons' (68) medium. Fermentation of glucose also occurred in the presence of CO_2 when this organism was cultivated in the medium described by Anderson and Ordal (4) at pH 8; in a phosphate buffered medium at pH 7.2; and, in the anaerobic medium minus the bicarbonate at pH 7.2. These data differ from the findings of Anderson and Ordal

(4) which indicate that CO_2 is required by this organism for anaerobic glucose fermentation. It is possible that the strain of Cytophaga succinicans studied in this investigation is a mutant which has lost the CO_2 requirement. Only one culture, strain 35, was found to require CO_2 in order to initiate glucose fermentation. In this regard this culture could have a metabolic pathway similar to that of Cytophaga succinicans as described by Anderson and Ordal (4).

The ability to hydrolyze macromolecules appears to be characteristic of the entire group of myxobacteria. In this investigation a relatively wide range of macromolecules were attacked. Tributyrin was hydrolyzed by all of the isolates while 97% were able to decompose casein, gelatin and aesculin and 94% attacked starch. None of the isolates were able to utilize cellulose powder although 71% were able to attack carboxymethyl cellulose. Only 17% of the isolates were able to degrade chitin.

The results of the other physiological tests were found to be consistent with Porter's (68) findings as well as those found in the species descriptions given in Bergey's Manual of Determinative Bacteriology (12) and published species descriptions in the literature. All of the cultures were found to be catalase positive. None, however, possessed the respiratory enzyme cytochrome oxidase which Porter (68) reported present in 12 of the 41 isolates she examined. Porter (68) also reported that none of these same 41 cultures were able to produce ammonia from arginine. The present investigation

showed 74% of the isolates possessed arginine deaminase, the enzyme necessary for this reaction.

Stanier (78) reported a strain of Cytophaga johnsonae to be an active denitrifier. This is currently the only report of a denitrifying myxobacterium. Two of the isolates, strains 19 and 20, studied during this investigation were found to be denitrifiers. These cultures differ from Stanier's in that his was a soil isolate while these were isolated from a fresh water environment.

The nutrition of some of the fruiting myxobacteria has been extensively studied and Dworkin (25) discusses the findings of various investigators with regard to both nitrogen and carbon requirements of these organisms. Until 1940 soil cytophagas were felt to have a very limited nutrition in that they were considered to be obligate cellulose-decomposing bacteria that would not grow in the absence of this substrate. At this time Stanier's (79) discovery of marine cytophagas with much less restricted nutritional requirements lead to the assumption that the carbon nutrition of soil forms might be broader than previously thought. In a 1942 report, Stanier (75) discusses the carbon and nitrogen requirements of soil cytophagas and indicates that growth occurs at the expense of cellobiose and glucose in addition to cellulose when the carbohydrates are filter sterilized. In some cases mannose and xylose could also be used. These results thus disposed of the biochemical anomaly of "obligate" cellulose

decomposition by cytophagas. However, the range of available carbon sources for these organisms appeared to be somewhat narrow, indicating a considerable degree of specialization. The data also indicated maximum growth of Cytophaga strains in cellulose in the presence of inorganic nitrogen in the form of $(NH_4)_2SO_4$ and KNO_3 and organic nitrogen in the form of asparitic acid and peptone. It was also noted that monoamino monocarboxylic amino acids (glycine, alanine and leucine) could not be utilized as nitrogen sources.

While no attempt was made to determine the precise nutritional requirements of the fresh water isolates, it was found that both casein hydrolysate and KNO_3 could serve as sources of nitrogen for all of the organisms when starch was used as the carbon source. When either ammonia or nitrite were used as the nitrogen sources the number of isolates able to initiate growth decreased. Approximately 30% of the isolates were able to utilize either glucose or cellobiose as a sole carbon source when nitrate was used as the nitrogen source. Citrate, as a sole carbon source, was utilized by three of the isolates. None of the amino acids tested was found to satisfy the carbon requirement.

Numerous reports (27, 34, 39, 47, 53, 62, 63, 65, 70, 83, 85, 90) are available concerning the ability of myxobacteria to attack and lyse various living microorganisms. Myxobacteria can also be cultivated on autoclaved cells which they digest by a proteolytic enzyme (83). An exoenzyme, amidase, which lyses intact cells, heat

killed cells and isolated cell walls of Arthrobacter crystalloposites has been obtained from a Cytophaga species (27). This enzyme disintegrates the cell wall mucopeptides into oligosaccharides and peptide fragments.

Generally, the literature indicates that myxobacteria more easily lyse, and grow more readily, on gram negative rather than on gram positive organisms. The six gram negative dead cell bacterial preparations examined in this investigation were found to be lysed by 77 to 100% of the isolates. Of the seven gram positive dead cell bacterial preparations used, four (Bacillus subtilis, Streptococcus faecalis, Staphylococcus aureus and Mycobacterium smegmatis) were shown to be lysed by less than 3% of the isolates. The same four cultures in a similar study by Porter (68) were lysed by 39 to 96% of the 41 isolates she examined. The reason for this discrepancy is not understood. In the case of Mycobacterium smegmatis it was noted that the cell preparation was made up of clumps of cells rather than the cells being evenly distributed throughout the medium. This may have obscured the actual presence of lysis if it occurred. The three remaining gram positive dead cell bacterial preparations examined were shown to be lysed by 80 to 91% of the isolates.

This investigation also suggests that yeast may be quite susceptible to lysis by myxobacterial organisms. A culture of Saccharomyces cerevisiae was found to be lysed by 80% of the

isolates. Algal and protozoan cells seemed to be more resistant to lysis by these organisms. Less than 6% of the cultures were able to lyse dead cell preparations of these organisms.

DNA base compositions are becoming more widely used in taxonomy and it is evident that there is a need to include such data into future species descriptions. In connection with a general taxonomic study of the 35 fresh water myxobacterial isolates, the DNA base compositions of six representative strains were determined.

The results of thermal denaturation curves suggested that cultures 4, 8, 25, 33 and 34 were closely related by their similarity of average Tm values (83.6 to 85.1C) and DNA base ratios (34.88 to 38.54% GC). The % GC obtained for culture 34, Cytophaga succinicans (RL-8), was found to be 38.54% which is in close agreement with the published value of 38% for this culture (44). The values obtained for the other four cultures were well within the published range of 32 to 43% GC for non-fruiting myxobacteria. That these five strains possess many morphological, physiological and biochemical similarities as well as similar DNA base compositions suggests possible genetic affinities and perhaps a common phylogenetic origin.

Culture 20 did not appear to be as closely related to the other five cultures examined. The GC ratio of this strain was found to be 52.44%. While this value is higher than reported for most Cytophaga

species, it should be noted that DeLey and van Muylen (24) have published values of 62.9 and 63.7% GC for two strains of swarming bacteria which they report as Cytophaga species. The % GC value obtained for strain 20 differed by at least 15% from that of any of the other five cultures studied. These findings would indicate, as noted by Sueoka (84), that the strain has few if any DNA base sequences in common with the other five strains. Thus they are probably not phylogenetically related. Taking into account the DNA base ratio obtained as well as cultural, physiological and biochemical reactions, it is felt that a more intensive study of this particular culture is in order.

Members of the order Myxobacterales are usually pigmented yellow, orange, pink or olive green. While Dworkin (25) states that diffusible pigments are not produced, one of the divisions found in the key to the genus Cytophaga (12) is based on the presence or absence of a "diffusible black to brown pigment." The pigments present in myxobacteria were suggested to be carotenoids by Jahn (25). Subsequent analysis by Greene and Leadbetter (35, 36, 37) as well as Burchard and Dworkin (13) have confirmed this.

While the carotenoids of myxobacterial organisms have remained relatively uncharacterized, information is available concerning the presence of these compounds in Cytophaga strains as revealed by chemical tests. Anderson and Ordal (4) used chemical

means to determine the carotenoid nature of the yellow pigment associated with the cell wall of Cytophaga succinicans. Fox and Lewin (28), in a preliminary study, attempted to characterize the carotenoids of some Flexibacteria in hopes of obtaining comparative biochemical information that would be of help in evaluating possible affinities occurring among these organisms. It was found that the general carotenoid metabolism of these organisms showed some similarities to blue-green algae and to certain eubacteria, notably in the absence of lutein and in the presence of largely xanthophyllic carotenoids.

The results of the pigment analysis carried out in this investigation suggest that four of the six isolates examined share a common yellow pigment which has an absorption spectrum similar to but not identical to lutein. Three of these same cultures also appeared to have a second yellow-orange pigment in common which was spectrally similar to the xanthophyll alpha-carotene-5, 6-epoxide. One of the cultures, strain 33, possessed a pale pink pigment, which appeared to be spectrally similar to the carotenoid rhodopin. As noted in the present study some differences in pigment make-up do occur among the myxobacterial isolates. Thus it is assumed that the use of information obtained from thin layer chromatography and spectral analyses should prove useful in supplementing myxobacterial species descriptions providing additional studies are carried out under standardized conditions.

The information obtained as a result of this investigation has been used to develop a new taxonomic key for the genus Cytophaga. This proposed scheme is presented in Table 18. The major divisions are based on the characteristics of carboxymethyl cellulose and chitin utilization as well as aerobic nitrate reduction and citrate utilization. Each of these categories can then be subdivided on the basis of additional physiological and biochemical reactions.

It seems pertinent to note that a reexamination of the data obtained by Porter (68) for 41 myxobacterial isolates obtained from fish showed that these cultures also fall within the frame-work of this proposed key. Since none of the 41 isolates examined by Porter were found capable of utilizing carboxymethyl cellulose by the method employed in that investigation these isolates would enter into the key at division AA. Carboxymethyl Cellulose Not Utilized. However, if these cultures were reexamined for carboxymethyl cellulose utilization using the method employed in this investigation, it is probable that a number would be found capable of utilizing this substrate and thus would enter the key at a higher position.

Very little physiological or cultural data are given for the species of the genus Cytophaga that are included in Bergey's Manual of Determinative Bacteriology (12). As a result it is difficult to determine where these organisms would be placed in the proposed key. It would be of interest to examine in more detail the cultural and

physiological characteristics of the species included in the genus Cytophaga so that these species could be more readily compared with the organisms studied in the present investigation.

It should be noted that in the present investigation all of the isolates capable of utilizing chitin also utilized carboxymethyl cellulose. Thus the first division made under Carboxymethyl Cellulose Not Utilized (i. e., Chitin Utilized) was made to accommodate the results obtained by Porter (68). Should a reexamination of Porter's isolates reveal that all chitin utilizers also utilize carboxymethyl cellulose, the second major division in this key may be further divided on the basis of citrate utilization and aerobic nitrate reduction rather than chitin utilization.

The proposed key differs from that provided in Bergey's Manual of Determinative Bacteriology (12) as well as that proposed by Porter (68) in that the major divisions have been based on physiological and biochemical reactions. Habitat and pigmentation were given priority over physiological and biochemical reactions in the two previously mentioned keys. It seems more reasonable to use habitat and pigmentation in the species descriptions following such a key rather than using these criteria as major characteristics for separating species, particularly until more intensive studies are carried out in that area.

While the proposed taxonomic scheme has a number of advantages over the key to the genus Cytophaga provided in Bergey's

Manual of Determinative Bacteriology (12) additional studies are needed. A more complete examination of the cultural, physiological and biochemical properties of soil and marine cytophagas is needed. This information should enable a more complete and useful key to this group of organisms to be devised. Serological investigations as well as DNA homology studies among members of the genus Cytophaga would also provide useful methods for studying additional inter-relationships among the members of this unique group.

Table 17. The Number and Percentage of Isolates Giving Positive Results in Tests.

	Number Positive*	Percent Positive
Fruiting Bodies Formed	0/35	0.0
Microcysts Formed	2/35	5.7
Growth at:		
5C	35/35	100.0
8C	35/35	100.0
10C	35/35	100.0
18C	35/35	100.0
27C	35/35	100.0
30C	31/35	88.6
37C	0/35	0.0
45C	0/35	0.0
55C	0/35	0.0
Growth at pH:		
5.0	0/35	0.0
5.5	0/35	0.0
6.0	27/35	77.1
6.5	29/35	82.9
7.0	33/35	94.3
7.2	35/35	100.0
7.5	26/35	74.3
8.0	25/35	71.4
9.0	16/35	45.7
Growth in NaCl:		
0.0%	35/35	100.0
0.2%	33/35	94.3
0.4%	31/35	88.6
0.6%	27/35	77.1
0.8%	26/35	74.3
1.0%	26/35	74.3
3.0%	0/35	0.0
Anaerobic Growth:		
Unbuffered Medium	5/35	14.3
Buffered Medium	25/35	71.4
Medium Minus $NaHCO_3$	33/35	94.3
Sensitivity to Antibiotics:		
Tetracycline	35/35	100.0
Streptomycin	35/35	100.0
Penicillin G	10/35	28.6
Bacitracin	10/35	28.6
Novobiocin	31/35	88.6

Table 17. (Continued)

	Number Positive*	Percent Positive
Erythromycin	30/35	85.7
Gantrisin	11/35	31.4
Neomycin	1/35	2.9
Polymyxin B	4/35	11.4
Heat Resistance:		
a. Young Cells		
Number viable after		
5 minutes at 37C	33/35	94.3
15 minutes at 37C	33/35	94.3
5 minutes at 55C	28/35	80.0
15 minutes at 55C	27/35	77.1
b. Old Cells		
Number viable after		
5 minutes at 50C	29/35	82.9
15 minutes at 50C	28/35	80.0
5 minutes at 70C	0/35	0.0
15 minutes at 70C	0/35	0.0
Carbohydrate Utilization:		
Glucose Oxidation	30/35	85.7
Glucose Fermentation	5/35	14.3
Lactose Oxidation	17/35	48.6
Lactose Fermentation	4/35	11.4
Sucrose Oxidation	13/35	37.1
Sucrose Fermentation	4/35	11.4
Galactose Oxidation	28/35	80.0
Galactose Fermentation	6/35	17.1
Maltose Oxidation	29/34	85.3
Maltose Fermentation	5/34	14.7
Cellobiose Oxidation	28/35	80.0
Cellobiose Fermentation	4/35	11.4
Mannitol Oxidation	1/34	2.9
Mannitol Fermentation	0/34	0.0
CO_2 Required for Glucose Fermentation	1/35	2.9
Degradation of Macromolecules:		
Starch	33/35	94.3
Casein	34/35	97.1
Gelatin	34/35	97.1
Tributyrin	35/35	100.0
Aesculin	34/35	97.1
Cellulose Powder	0/35	0.0
Carboxymethyl Cellulose	25/35	71.4

Table 17. (Continued)

	Number Positive*	Percent Positive
Chitin	6/35	17.1
Miscellaneous Physiological Tests:		
Hydrogen Sulfide	4/35	11.4
Nitrate Reducation:		
Aerobically	22/35	62.9
Anaerobically	17/35	48.6
Aerobically with Gas	2/35	5.7
Anaerobically with Gas	2/35	5.7
Denitrifier	2/35	5.7
Urease	0/35	0.0
Lysine Decarboxylase	0/35	0.0
Arginine Deaminase	26/35	74.3
Tyrosine Hydrolysis	9/35	25.7
Proteolytic Action on Litmus Milk	30/35	85.7
Indol	0/35	0.0
Methyl Red	0/35	0.0
Voges-Proskauer	0/35	0.0
Catalase	35/35	100.0
Cytochrome Oxidase	0/35	0.0
Nutritional Studies:		
a. Sole Nitrogen Source		
Casein Hydrolysate	35/35	100.0
$(NH_4)_2SO_4$	26/35	74.3
KNO_3	35/35	100.0
KNO_2	15/35	42.8
b. Sole Carbon Source		
Glucose	12/35	34.3
Cellobiose	13/35	37.1
Citrate	3/35	8.6
Sodium Bicarbonate	0/35	0.0
Arginine	0/35	0.0
Cysteine	0/35	0.0
Alanine	0/35	0.0
Glycine	0/35	0.0
Glutamic Acid	0/35	0.0
Proline	0/35	0.0
Lysis of Dead Cells:		
Anabaena sp.	2/35	5.7
Scenedesmus obliqus strain D_3	1/35	2.9

Table 17. (Continued)

	Number Positive*	Percent Positive
Arthrobacter sp.	31/35	88.6
Corynebacterium hofmanii	31/35	88.6
Alcaligenes viscolactis	27/35	77.1
Sarcina ureae	32/35	91.4
Pseudomonas aeruginosa	34/35	97.1
Bacillus megatherium	28/35	80.0
Bacillus subtilis	0/35	0.0
Aerobacter aerogenes	35/35	100.0
Escherichia coli	33/35	94.3
Streptococcus faecalis	0/35	0.0
Staphylococcus aureus	1/35	2.9
Mycobacterium smegmatis	0/35	0.0
Proteus vulgaris	33/35	94.3
Euglena gracilis	1/35	2.9
Saccharomyces cerevisiae	28/35	80.0
Number of Isolates Susceptible to Infection by ϕ-173-N	10/35	28.6

*The numerator indicates the number of isolates positive for a test and the denominator indicates the total number of isolates tested.

Table 18. Proposed Taxonomic Key for Thirty-five Fresh Water Myxobacterial Isolates.

I. Resting Cells Produced. Fruiting Bodies Absent. Genus Sporocytophaga.
- A. Denitrifier.
  - 1. Isolate 20
- AA. Not a Denitrifier.
  - 2. Isolate 4

II. Resting Cells Not Produced. Fruiting Bodies Absent. Genus Cytophaga.
- A. Carboxymethyl Cellulose Utilized.
  - 1. Chitin Utilized.
    - a. Nitrate reduced aerobically.
      - b. Lactose oxidized.
        - 3. Isolate 8
      - bb. Lactose not oxidized.
        - c. Sucrose oxidized.
          - 4. Isolate 32
        - cc. Sucrose not oxidized.
          - d. Maltose oxidized.
            - 5. Isolate 10
          - dd. Maltose not oxidized.
            - 6. Isolate 13
    - aa. Nitrate not reduced aerobically.
      - b. Glucose, galactose and cellobiose oxidized.
        - 7. Isolate 11
      - bb. Glucose, galactose and cellobiose not oxidized.
        - 8. Isolate 29
  - 2. Chitin Not Utilized.
    - a. Citrate utilized.
      - b. Nitrate reduced aerobically.
        - 9. Isolate 14
      - bb. Nitrate not reduced aerobically.
        - 10. Isolate 26
    - aa. Citrate not utilized.
      - b. Nitrate reduced aerobically.
        - c. Nitrate reduced anaerobically.
          - d. Glucose oxidized.
            - e. Lactose oxidized.
              - f. Sucrose oxidized.
                - 11. Isolate 15
              - ff. Sucrose not oxidized

Table 18. (Continued)

g. Sensitive to Bacitracin; CO_2 dependent glucose fermentation.
12. Isolate 35
gg. Not sensitive to Bacitracin.
13. Isolate 2
ee. Lactose not oxidized.
h. Galactose oxidized.
i. Mannitol oxidized.
14. Isolate 12
ii. Mannitol not oxidized.
15. Isolate 17
hh. Galactose not oxidized.
16. Isolate 27
dd. Glucose not oxidized; Glucose fermented.
j. Arginine deaminated.
17. Isolate 31
jj. Arginine not deaminated.
18. Isolate 24
cc. Nitrate not reduced anaerobically.
k. Hydrogen sulfide produced.
19. Isolate 3
kk. Hydrogen sulfide not produced.
l. Tyrosine hydrolyzed.
20. Isolate 7
ll. Tyrosine not hydrolyzed.
m. Sucrose oxidized.
21. Isolate 9
mm. Sucrose not oxidized.
22. Isolate 18
bb. Nitrate not reduced aerobically.
n. Glucose oxidized.
o. Sucrose oxidized.
23. Isolate 25
oo. Sucrose not oxidized.
p. Lactose oxidized; Not sensitive to Bacitracin.
24. Isolate 1
pp. Lactose not oxidized; Sensitive to Bacitracin.
25. Isolate 5
nn. Glucose not oxidized.
26. Isolate 33

Table 18. (Continued)

AA. Carboxymethyl Cellulose Not Utilized.
1. Chitin Utilized.
2. Chitin Not Utilized.
a. Citrate utilized.
27. Isolate 22
aa. Citrate not utilized.
b. Nitrate reduced aerobically.
c. Denitrifier; Gas produced during aerobic nitrate reduction.
28. Isolate 19
cc. Not a denitrifier; Gas not produced during aerobic nitrate reduction.
d. Tyrosine hydrolyzed; Sucrose not fermented.
29. Isolate 34
dd. Tyrosine not hydrolyzed. Sucrose fermented.
30. Isolate 23
bb. Nitrate not reduced aerobically.
e. Glucose oxidized.
f. Lactose oxidized.
g. Tyrosine hydrolyzed.
31. Isolate 21
gg. Tyrosine not hydrolyzed.
32. Isolate 6
ff. Lactose not oxidized.
33. Isolate 30
ee. Glucose not oxidized.
f. Sensitive to neomycin; Pigment not enhanced by tyrosine.
34. Isolate 28
ff. Not sensitive to neomycin; Pigment enhanced by tyrosine.
35. Isolate 16

SUMMARY

1. The 35 fresh water isolates examined in this investigation were initially identified as members of the order Myxobacterales on the basis of their morphology and gliding motility as ascertained through the use of phase contrast microscopy. These isolates were subsequently examined in an attempt to determine additional cultural, physiological and biochemical characteristics.

2. None of the isolates were observed to form fruiting bodies. Two, however, appeared to form microcysts and have been tentatively designated members of the genus Sporocytophaga. The remaining 33 isolates form neither fruiting bodies nor microcysts and consequently are classified in the genus Cytophaga.

3. DNA base composition analysis of five of these isolates showed them to be closely related. All had values well within the published range of % GC for non-fruiting myxobacteria.

4. The pigments associated with these isolates appear to be carotenoid in nature. In the five isolates examined, a common yellow pigment was noted in cultures that were yellow or yellow-orange in color. In cultures that appeared to be more orange in color, a second pigment was encountered. The pigments associated with one of the isolates were significantly different than the others in that it was pale pink in color. All of these pigments were found to be

spectrally similar but not identical to known carotenoids.

5. Based on the cultural and physiological characteristics of the organisms studied, a new taxonomic scheme for the genus Cytophaga has been proposed.

6. A bacteriophage was isolated during this investigation that has a rather wide host range. Complete characterization studies of this phage were not carried out.

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APPENDIX

Media

Components of media used in this investigation are listed below. Formulas are given in percentage (w/v). Adjustments in pH were made using 1N NaOH and 0.1N or 1N HCl.

Anaerobic Medium

Difco peptone	0.18%
Difco yeast extract	0.09%
Difco beef extract	0.09%
Filtered glucose	0.9%
Filtered sodium bicarbonate	0.18%

pH 7.2

Arginine Broth

Casitone	1.5%
K_2HPO_4	0.1%
Glucose	0.1%
L-arginine monohydrochloride	0.5%

pH 7.2

Basal Medium for Urease Detection

Difco peptone	0.1%
NaCl	0.3%
KH_2PO_4	0.1%

pH 7.2

Casein Hydrolysate Broth

$NH_4H_2PO_4$	0.1%
KCl	0.02%
$MgSO_4 \cdot 7H_2O$	0.02%
Casein hydrolysate	0.02%

pH 7.2

Chitin Agar

Difco peptone	0.1%
Chitin	0.5%
Difco agar	1.0%

pH 7.0 to 7.2

Cytophaga Media

Broth:	Difco tryptone	0.05%
	Difco yeast extract	0.05%
	Difco beef extract	0.02%
	Sodium acetate	0.02%

pH 7.0 to 7.2

Agar: Cytophaga broth plus 1.1% (w/v) Difco agar.

Agar Deeps: Cytophaga broth plus 0.4% (w/v) Difco agar.

0.7% Cytophaga Agar: Cytophaga broth plus 0.7% (w/v) Difco agar.

1/10 Cytophaga Peptonized Milk Agar (CPM)

Difco tryptone	0.005%
Difco yeast extract	0.005%
Difco beef extract	0.002%
Sodium acetate	0.002%
Peptonized milk	0.05%
Difco agar	1.5%

pH 7.2

Ferric Ammonium Citrate Medium (Aesculin Medium)

Difco peptone	1.0%
Ferric ammonium citrate	0.05%
Difco aesculin	0.1%
Difco agar	1.5%

pH 7.2

Koser's Citrate Medium

K_2HPO_4	0.1%
$MgSO_4 \cdot 7H_2O$	0.02%

$NaNH_4PO_4$	0.15%
$Na_3C_6H_5O_7$	0.3%

pH 7.2

Lysine Decarboxylase Medium

Pancreatic digest of casein	1.5%
Filtered glucose	0.1%
K_2HPO_4	0.1%

pH 7.2

Mineral Salts Medium

$MgSO_4 \cdot 7H_2O$	0.02%
K_2HPO_4	0.01%
$CaCl_2 \cdot 2H_2O$	0.002%
$MnCl_2 \cdot 4H_2O$	0.0002%
$NaMoO_4 \cdot 2H_2O$	0.0001%
$FeSO_4 \cdot 7H_2O$	0.0001%
NH_4Cl	0.05%

pH 7.2

Modified Carboxymethyl Cellulose Medium

Difco peptone	0.1%
Difco agar	1.0%
Carboxymethyl cellulose-sodium salt	1.5-2.0%
Bromthymol blue	0.0015%

pH 7.2

Modified Clark-Lub Medium

Difco peptone	0.7%
Filtered glucose	0.5%
K_2HPO_4	0.1%

pH 7.2

Modified Denitrification Medium

Difco beef extract	0.3%
Difco peptone	0.5%
KNO_3	0.3%

Glycerol		0.5%
Difco agar		0.4%
	pH 7.2	

Modified Hugh-Leifson's Medium

Difco peptone		0.2%
K_2HPO_4		0.03%
Difco agar		0.3%
Filtered carbohydrate		0.5%
Bromthymol blue		0.0015%
	pH 7.0 to 7.2	

Modified Nitrate Broth

Difco peptone		0.5%
Difco beef extract		0.3%
KNO_3		0.1%
	pH 7.2	

Peptonized Milk Agar (PMA)

Peptonized milk		0.1%
Difco agar		1.5%
	pH 7.2	

Stanier's Mineral Basal Medium

$(NH_4)_2SO_4$ or KNO_3		0.1%
K_2HPO_4		0.1%
$MgSO_4 \cdot 7H_2O$		0.02%
$CaCl_2 \cdot 2H_2O$		0.01%
$FeCl_3$		0.002%
Tap water		
	pH 7.0 to 7.2	

Tryptone-Calcium Chloride Media

Broth:	Difco tryptone		0.5%
	$CaCl_2 \cdot 2H_2O$		0.002%
		pH 7.2	

Agar: Broth plus 1.5% (w/v) Difco agar.

0.7% Tryptone-Calcium Chloride Agar: Broth plus 0.7% (w/v) Difco agar.